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Symposium on Coxsackie Group of Viruses[1]

Chairman: GILBERT DALLDORF

THE COXSACKIE VIRUSES

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IT SEEMS TO BE SOUNDLY ESTABLISHED that what we have called the Coxsackie viruses are common infections of man. The speakers that follow me will discuss the typing of various strains of Coxsackie viruses, their behavior in complement-fixation tests, their isolation under various circumstances and something of the nature of the human disease with which they have been associated (1).

It will be well to bear in mind that we are sadly deficient in our understanding of the human disease and know nothing regarding the lesions, if any, in man. That is why it seemed unwise to give the virus a descriptive name.

It may prove to be significant that the strains we have studied so far can be readily classified in two groups on the basis of the experimental disease (2). This may easily and definitely be done by histologic examination. *Group A* includes strains that induce lesions only of the skeletal muscles. *Group-B* strains also cause lesions in the brain, sometimes in the fat pads, heart and other organs. We find the two kinds of Coxsackie virus differ in size and other respects. In our work it has been an advantage to be able to distinguish between the two and I suspect others will find it equally helpful.

So far these agents have been found associated only with man; in human feces and throat washings, in sewage and in flies trapped near human habitations. They have not been found as natural infections in other animals. They have commonly been associated with epidemics of poliomyelitis or poliomyelitis-like disease. The association of Coxsackie and poliomyelitis viruses deserves attention and poses several interesting questions.

Our experience in New York during the past 3 years has been that Coxsackie virus may sometimes be found in the feces of poliomyelitis patients, sometimes alone, sometimes in combination with poliomyelitis virus. The data we have indicate that infection has distinct epidemiologic patterns, is largely limited to the young (the age distribution is similar to that of poliomyelitis some years ago), and is associated with symptoms indistinguishable from those of poliomyelitis. At any rate, we have not been able to distinguish between the symptoms and signs in the patients from whom we can and cannot isolate Coxsackie or poliomyelitis virus. This may simply mean that the Coxsackie viruses flourish during the summer months and have little clinical effect, that the disease we see is due in large measure or entirely to poliomyelitis virus.

Others have found Coxsackie virus associated with outbreaks of diseases of a different kind. It may be noted that so far these reports have been of *group-B* virus infections. Dr. Kilbourne will describe such an outbreak. The observations of Curnen, Shaw, and Melnick have much in common. We should look for Coxsackie viruses in several diseases for they seem to be associated with diverse clinical conditions.

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[1] Atlantic City, N. J., April 19, 1950.

COMPLEMENT-FIXATION TESTS WITH SOME OF THE VIRUSES IN THE COXSACKIE GROUP[1]

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AN EARLY INTEREST of the writers concerned possibilities of laboratory diagnosis of the viruses comprised in the group designated by Dalldorf (1) as Coxsackie. While other members of the Symposium will discuss various methods of detection and identification of the viruses, this presentation is limited to complement-fixation tests. For the purposes of the study 4 laboratory strains or viruses of this group have been used: *type 1* (Dalldorf) (2, 3), *type 2* (Dalldorf) (2, 3), Conn. 5 (4) and 1135K (5). Other colleagues of the Symposium will hold forth on the problem of the taxonomy of these agents: whether they are distinct, independent viruses or whether they are strains of the same virus. It is, however, of considerable interest that the viruses here employed have dissimilar properties in the experimental animal and that *type 1* was isolated from the feces of a child with a diagnosis of paralytic poliomyelitis, *type 2* and Conn. 5, from stools of patients considered as cases of nonparalytic poliomyelitis, and 1135K, from the throat washings of a patient having an acute, febrile, influenza-like illness and whose chief complaint was abdominal pain (6). Only the future will tell whether one is dealing with a disease of protean manifestations, assuming a wide variety of clinical syndromes or whether the illnesses encountered, from which the Coxsackie viruses have been recovered, are different nosological entities caused by different viruses. From the results of the present investigations it would appear that, conforming with the designations commonly made by Dalldorf (1, 2), Curnen (6), Melnick (4) and others, the use of the term 'group of viruses' would at this time be preferable.

These facts are mentioned because they have bearing on the subject of complement fixation as a test for detection of specific antibody in the serum of individuals or for identification of a particular virus. An antigen used in such tests primarily *a*) should be free from any nonspecific reactions since so many agents, be they strains or different viruses, have already been recovered, *b*) should have a maximal titer, i.e. should be powerful enough to detect the smallest quantity of specific antibody, and *c*) should be practical and simple, easy to adapt to ordinary laboratory equipment or field conditions and to available technical assistance. It is believed that such an antigen can readily be prepared; its preparation and the details of a complement-fixation test with it has already been described (7).

Several reports have appeared since then by others describing a diversity of methods for performing this test with Coxsackie viruses. Thus Howitt and Benefield (8) have used unfractionated suspensions of infected, isolated muscle repeatedly frozen and thawed. Melnick and coworkers (4, 9) have devised antigens prepared by high-speed centrifugation and precipitation by protamine combined with a microtest performed on lucite plates, the readings being plotted and the area under each curve determined by means of a planimeter. Recently Manire, Sulkin and Farmer (10) have described antigens made from suspensions of infected mouse muscle either uncentrifuged or as supernate fluid after centrifugation at 2500 rpm for 30 minutes.

The method adopted for the preparation of antigens now used in this laboratory for the Coxsackie viruses, and the details of the procedure of the test are those that have already been applied to neurotropic viruses in general (11). The preparation of an antigen is based on successive extractions of infected tissue with acetone and ethyl ether; after drying, the final residue is suspended in saline solution to a concentration of 3:1 with respect to the original wet-tissue weight. The use of acetone-ether extraction is considered necessary since known Wassermann-positive sera react with normal, nonextracted

[1] We are grateful to Major L. C. Murphy, V. C., U. S. Army and Professor Italo Archetti of our laboratory and to Drs. G. Dalldorf, E. D. Kilbourne and R. M. Taylor for their valuable cooperation.

tissue, thereby giving rise to false positives. Following extraction, however, nonspecific reactions are eliminated in most instances or they are reduced to such a degree that a practical specific test results (table 1). The tissue from which the antigen is made is obtained from Swiss-W, infant mice which are etherized to death. Either the muscle can be stripped off the bones, which is difficult, or the entire limb can be used which is a simpler method and yields as good results. Brain tissue, owing probably to a low content of virus, does not produce effective antigens.

By this method antigens can readily be obtained with *type 1* and *2* from infected mice up to 14 to 15 days of age at the time of infection; best results, however, follow the injection with virus of 6- to 7-day-old animals, for the tissue of such mice yields an antigen of higher titer which exhibits least anti-complementary effects. In addition, mice are injected intraperitoneally and infected tissue harvested on the third or fourth day.

On the other hand, mice are relatively less susceptible to the effects of either Conn. 5 or 1135K viruses. In these instances, animals 1, 2 or 3 days old are needed, for older ones either fail to be affected or develop mild illness from which they can recover. With the limb tissue of such mice an effective antigen can be prepared with Conn. 5 virus. Up to the present time, and work is still in progress, no usable antigen has been obtained with the 1135K virus. The probability is that a sufficiently large amount of virus per unit weight of tissue has not yet been secured. Antiserum against this virus can, however, be readily prepared so that a one-way test can be performed, i.e. a test to reveal antibody in 1135K antiserum against antigens other than 1135K. The antiserum is then found to contain antibody reactive against Conn. 5 virus but not against *types 1* and *2*. In addition, *types 1* and *2*, and Conn. 5 each appears to be antigenically specific (table 2, which shows a test with mouse antiserum). The relationship of 1135K virus to that of Conn. 5 is further discussed by Dr. Kilbourne, a member of this Symposium, who first isolated the former active agent.

Immune sera to be used as positive controls for testing antigens are prepared by the repeated intraperitoneal injections of 0.5 cc. of 10^{-2} dilution of suspensions of infected-limb tissue into 50- to 60-day-old mice. The inoculations are repeated 4 times at 3-day intervals, and animals are bled for serum collection 7 to 10 days after the last injection. The other details of preparation of materials for the complement-fixation tests have already been described (12). Table 2 offers an example of the results of several similar tests.

On the basis of the complement-fixation test

TABLE 1. REACTION OF HUMAN WASSERMANN-POSITIVE SERA WITH ACETONE-ETHER EXTRACTED AND NON-EXTRACTED ANTIGENS

SERUM	ANTIGEN												SALINE CONTROL
	Acetone-ether extracted Albany *type 2*						Nonextracted normal tissue						
Serum dilution reciprocal	2	4	8	16	32	64	2	4	8	16	32	64	2
Serum no. 1	2	2	0	0	0	0	4	4	4	3	2	0	0
2	±	0	0	0	0	0	4	4	4	±	0	0	0
3	1	±	0	0	0	0	4	4	4	4	4	1	0
4	2	1	0	0	0	0	4	4	3	2	0	0	0
5	1	0	0	0	0	0	4	2	2	2	0	0	0
6	0	0	0	0	0	0	2	2	±	0	0	0	0
7	0	0	0	0	0	0	4	4	2	0	0	0	0

TABLE 2. A COMPLEMENT-FIXATION TEST WITH COXSACKIE VIRUS

ANTIGEN AND TISSUE	SERUM			
	type 1	*type 2*	Conn. 5	1135K
Type 1 limb	1/64	0	0	0
Type 2 limb	0	1/64	0	0
Conn. 5 limb	0	0	1/64	1/64
Conn. 5 brain	0	0	0	0
1135K limb	0	0	0	0

as described here, 60 sera were studied which were obtained from 28 human beings, several of whom were bled more than once. The individuals from whom specimens were secured fell into 3 groups: *1*) laboratory contacts, i.e. those exposed during the course of handling the viruses; *2*) patients with various illnesses now suspected as being of the Coxsackie types and *3*) persons having no recollection of such illness or of contact with the virus. Of the first group, viz. laboratory contacts, there were 7; the serum of all was positive: all reacted with *type 1*, 6 also against Conn. 5, and 4 also against *type 2* antigens. As already stated no satisfactory antigen of the 1135K agent is as yet available. The results are interesting in view of the fact that the personnel of the laboratory was exposed to all of the 4 viruses, and most active work being carried on with *type 1*. The second group comprised persons from a wide geographic distribution. Our thanks are due Dr. R. M. Taylor for the specimens from Iceland, to Miss B. F. Howitt for the ones from Texas and to Dr. F. P. Nagler for those from Prince Edward Island, Canada. Of the serum of 18 individuals, 11 were tested with *type 1*, *2* and Conn. 5 antigens: one was positive with all of them, 4 with *type 1* and Conn. 5 only, 5 with *type 1* only and the eleventh was negative with all 3. Seven were tested with *type 1* and *2* antigens: 5 were positive with the former and all negative with the latter. It is noteworthy that complement-fixation antibody against *type 1* virus is generally more commonly met with here than against the others. The third group consisted of persons who had no history of illness suspected as being the Coxsackie types nor known contact with the virus. Of these the serum of only 3 could be collected for study: none reacted with *type 2*, one with *type 1* only, one with *type 1* and Conn. 5, and the third, with none of the 3 antigens. In table 3 is shown a series of selected 8 cases which are illustrative of the results obtained with the aforementioned 3 groups of tests.

From the results of the present studies it appears therefore that the complement-fixation test performed under controlled conditions offers a means for determining the presence of antibody in sera of man and experimental animals and for measuring its relative amount. The results were generally confirmed in later reports by others.

TABLE 3. COMPLEMENT FIXATION WITH HUMAN SERA. COXSACKIE-VIRUS ANTIGENS

NO.	SERUM History	ANTIGEN Albany *type 1*	Albany *type 2*	Conn. 5	Normal tissue
1	Laboratory contact with 3 strains	1:4	0	1:32	0
2	Laboratory contact with 3 strains	1:16	1:8	1:16	0
3	Laboratory contact with 3 strains	1:16	1:8	1:16	0
4	Clinical infection:				
	a) before onset	1:2	0	0	0
	b) month after	1:8	0	1:2	0
5	Clinical infection:				
	a) day of onset	1:2	0	0	0
	b) 2 months after	1:4	0	1:8	0
	c) 1 year after	1:2	0	1:2	0
6	No contact or recognized infection	1:4	0	0	0
7	No contact or recognized infection	1:8	0	1:8	0
8	No contact or recognized infection	0	0	0	0

The pattern of antibody level in several neurotropic virus infections runs along the following lines: neutralizing antibody appears, as a rule, early after exposure to a virus, rapidly reaches a high level and then either remains on a higher or lower plateau for a considerable period of time, perhaps for the life of the individual. With the complement-fixing antibody, however, the course is often different. Here, the antibody arises somewhat later, reaches promptly its highest level and then, in a matter of months, slowly diminishes to complete extinction. Whether the same pattern of antibody production is followed by the Coxsackie viruses cannot be determined at the present time.

It will be observed that in certain instances in which paired sera were available, the complement-fixing antibody showed an increase cor-

related with the course of the infection, after the manner of a specific antibody response. At the same time several specimens exhibited positive reaction to more than one antigen. In view of the specificity of the antigens as revealed by control tests with normal sera and prepared, known antisera, such reactions are considered to be essentially not cross reactions, but rather specific responses to the antigens in question.

It is clear, as shown here and by the work of others, that the Coxsackie viruses are widely distributed and that infection with them is not uncommon. Many persons show positive reactions for serum antibody among whom are those who have no recollection of contact with the virus or of suspected illness. Infection is, moreover, easily contracted as noted by the large incidence of positive reactions in workers handling the virus, several of whom have been unaffected by illness during this period of contact; although it is difficult to state this as cause and effect since these persons may have been positive before they came in contact with the viruses (viz. Curnen (6) for several clinically recognizable laboratory infections). The situation in the case of Coxsackie viruses is one that has similarities with that of poliomyelitis virus in which a large percentage of apparently normal human beings sooner or later develop antibody.

Since subclinical infections with the Coxsackie viruses may be a common occurrence (2, 4, 8) and in view of the fact that such infection leaves its signature in antibody production, it is clear that an antibody test per se on a single sample of serum or even on repeated samples is not a means for diagnosis, unless it can be shown by examination of sera deriving from early stages of a suspected illness and from later convalescent periods that there has been a distinct rise in antibody which is correlated with the course of the disease.

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ISOLATION AND DIFFERENTIATION OF THE COXSACKIE GROUP OF VIRUSES

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DURING THE PAST YEAR AND A HALF, material has been received either from sporadic cases or from groups of patients showing ill-defined clinical pictures such as mild fevers, poliomyelitis-like, encephalitis-like, or influenza-like syndromes. At first it was thought (1) that these cases might have some relationship to Newcastle Disease of chickens but since this virus was never isolated and as it was shown that the positive neutralization tests were due to a non-specific heat labile factor in the human sera, (2) this idea was abandoned. Following the report by Dalldorf and Sickles, (3) on recovery of the Coxsackie group of viruses from human feces, (4, 5) all the available material on hand was inoculated into suckling mice. Coxsackie viruses were recovered from the feces and nasopharyngeal washings of many of these patients. Subsequently more specimens were examined from various sources, and strains have been recovered from 105 individuals in 10 different states, Alabama, Colorado, Delaware, Florida, Georgia, Louisiana, Oklahoma, South Dakota, Tennessee and Texas.

METHODS

1) For Isolation of Viruses. The fecal and oral specimens were prepared according to a previously described method (6). Twenty per cent suspensions of feces were made in distilled water and run in the angle centrifuge ½ hour at 13,000 rpm in the cold room. One thousand units of penicillin and 10 mg. of streptomycin were added for each ml. of the supernatant fluid and the mixtures were left at room temperature for ½ hour before inoculation of 3- to 4-day-old mice. Each animal received 0.01 ml. intracerebrally, 0.03 ml. intramuscularly and 0.03 ml. intraperitoneally of the treated feces. The oral washings were treated in the same manner, except that the antibiotics were reduced to ½ the quantity.

If the mice showed muscle paralysis or were prostrate after 3 or 4 days' incubation, they were killed and the skeletal muscles removed. Because a higher concentration of virus was found in the muscles, Seitz filtrates of the latter tissues were used for all future work.

2) Immune sera were produced by inoculation of mice, guinea pigs, hamsters, rabbits or monkeys with either brain or the filtered muscle suspensions. An effective serum was obtained in about 3 weeks by bi-weekly intravenous injections of rabbits with filtered infected mice muscles.

3) Neutralization tests were performed according to the method in the U. S. Army Laboratory Manual (7), except that the serum-virus mixtures were incubated for 20 minutes at 37° C. and held for 20 minutes in the cold room before inoculating 0.03 ml. intramuscularly into 3- to 4-day-old mice. All sera were inactivated at 56° C. for 30 minutes before use. Although the virus strains had LD_{50} titers of between $10^{-6.5}$ and $10^{-8.5}$, many undiluted sera gave such high protection that a neutralization index often was not obtained when only 2 or 3 dilutions of virus were used. In tests reported as positive, however, the serum neutralized at least 1000 LD_{50} doses of virus. Occasionally, the tests were performed on different dilutions of sera against a constant amount of virus, usually 100 LD_{50} doses.

4) The methods used for the complement-fixation tests have been recently described (8). Filtered mice muscles were used for antigens and the tests were performed according to a modified Kolmer technique with over-night fixation in the cold.

VIRUS ISOLATIONS

The first 5 viruses that gave symptoms of muscle paralysis in suckling mice were isolated from human feces at monthly intervals from October 1948, to January 1949. After these original isolations of the Coxsackie virus, a systematic study was made of all material remaining from the earlier collections beginning with 1947 and continuing through 1949. This material had been kept frozen at either −10° C. or −60° C. and in

[1] From the Virus and Rickettsia Section, Montgomery, Ala.

many instances the virus remained viable after storage for over 2 years at the latter temperature.

ings, mouth vesicles, nasal swabs, saliva, sputum, serum, whole blood, lung, cord and brain, but not spinal fluid that had been sent in at various

TABLE 1. SUMMARY OF ISOLATIONS OF COXSACKIE VIRUSES FROM HUMAN SOURCES[1]

STATE	ORAL WASHINGS		FECES		BLOOD		BRAIN OR CORD		SPINAL FLUID	
	No. tested	No. positive	No. tested	No. positive	No. tested	No. positive	No. tested	No. positive	No. tested	No. positive
Ala.	34	24	16	5	21	11	5	3		
Colo.			2	2			1	0		
Del.	48	7	31	9					20	0
Fla.	4	4	6	5	4	3				
Ga.	11	7	13	7	2	2				
La.	6	2	5	1	1	1	4	3		
Okla.	1	1	4	1	1	1				
S. D.							1	1		
Tenn.	16	5	6	1	10	8	4	2		
Tex.							1	1		
Totals	120	50 (41.6%)	83	31 (37.3%)	39	26 (66.6%)	16	10 (62.5%)	20	0

[1] Includes 2 sputum specimens.

TABLE 2. COXSACKIE VIRUS ISOLATIONS FROM FATAL CASES

CASE NO.	AGE	C	SEX	LOCATION	DATES OF		MATERIAL TESTED	DIAGNOSIS	TYPE OF VIRUS ISOLATED
					Onset	Death			
	yrs.								
1	56	C	M	Ala.	1/31/49	2/14/49	Brain	TB of lungs, Encephalomyelitis	2
2	4	W	F	Ala.	9/1/49	9/15/49	Spinal cord	Poliomyelitis Polio isolated	4[1]
3	2	W	M	Ala.	9/3/49	9/27/49	Spinal cord	Autopsy findings negative	4
4				Conn.	12/49	2/5/50	Lung	Pneumonitis	
5	6	W	M	Ga.	5/30/49	6/2/49	Serum	Encephalitis	2
6	2	C	M	La.	?	9/1/49	Serum	Poliomyelitis	2[1]
7	34	W	M	La.	8/3/49	8/11/49	Brain	Poliomyelitis or Encephalomyelitis	4[1]
8	12	W	M	La.	10/1/49	10/18/49	Brain	Encephalitis ?	5
9	20	W	F	S. D.	12/10/49	12/17/49	Brain	Poliomyelitis Encephalitis ?	4
10	3	W	M	Tenn.	7/15/48	7/25/48	Nasal washing	Encephalomyelopathy	4[1]
							Brain		4[1]
							Serum		4[1]
11	34	W	M	Tenn.	11/16/49	11/23/49	Brain stem	Poliomyelitis	2
12	?		F	Tex.	No information		Brain stem	Poliomyelitis	5

[1] Virus of the same type isolated a second time from the same material.

Coxsackie viruses have been isolated from human feces, nasopharyngeal or mouth wash-

times from scattered localities in 10 different states. Table 1 gives a summary of 117 isolations

and shows both the geographical distribution and the number obtained from the different kinds of specimens.

While it has been believed that the Coxsackie viruses are associated with a benign type of infection, it is interesting to observe, as shown in table 2, that they have been recovered from the oral washings of one, the lung of one, the serum of 2 and the nervous tissues of 9 fatal cases. Six of these 12 fatal cases were diagnosed as having poliomyelitis. Poliomyelitis and Coxsackie viruses were recovered simultaneously from the spinal cord of one of these patients. They were obtained also from the feces of 2 other non-fatal cases. It may be seen likewise that the same type of virus was recovered twice from 3 different materials (brain, nasal washings and serum) from the same patient.

Virus was obtained from either the clotted blood or the serum of 26 patients. All of the blood samples had been kept frozen at −10° C. and some were held for over a year. While 14 of the specimens were obtained during the first week of illness, one was taken in 9 and another in 13 days after the onset. The dates of onset were unknown for the other patients.

Specimens were received from a number of outbreaks which, although characterized by diverse clinical pictures, were all suspected of having a viral origin. The symptoms were usually those of a non-paralytic poliomyelitis or a mild 3- to 5-day fever accompanied by headache and often muscle pains. In one group of adults influenza was suspected. Because no influenza virus was isolated, the throat washings were tested for the Coxsackie strains with the results as shown in table 3.

The largest number of isolations has been from material of patients becoming ill during August and September, although specimens have been

TABLE 3. OUTBREAKS ASSOCIATED WITH ISOLATIONS OF COXSACKIE VIRUS

LOCATION	YEAR	GENERAL TYPE OF CASE	PREDOMINANT SYMPTOMS	TOTAL NO. OF CASES TESTED	VIRUS ISOLATIONS FROM					
					Feces		Mouth Garglings		Blood	
					No. tested	No. positive	No. tested	No. positive	No. tested	No. positive
Wilmington, Del.	1947	Poliomyelitis-like	Fever, headache, some muscle weakness	46	31	9	36	1		
							12[1]	6		
Enterprise, Ala.	1948	Poliomyelitis-like	3–5 day fever, headache, nausia, vomiting	19	6	2	5	2	7	6
Shreveport, La.	1948	Mild fever	3-day fever, headache, no neurological symptoms	5	4	1	6	2		
Nashville, Tenn.	1948	Encephalitis-like or atypical syndromes	Varied symptoms, headache, fever	17	3	0	13	3	10	8
Montgomery, Ala.	1949	Influenza-like	Fever, aches, some mouth vesicles	21			21	12	9	3
Swainsboro, Ga.	1949	Generalized Febrile	Fever, headache, vomiting, malaise	14	7	2	9	5	1	1

[1] Contact nurses.

positive from cases occurring throughout the year, especially in the spring. Curnen, Shaw and Melnick (9) have reported likewise that the virus has been more often recovered from the August and September specimens.

Differentiation into Serological Types. Early in these studies it was observed that the antiserum from one strain of virus did not afford protection against several others. When more viruses were isolated, it became apparent that they belonged to serologically distinct types. The presence of a multiplicity of strains has been reported by both Dalldorf *et al.* (3–5) and Melnick *et al.* (10). Through the courtesy of Dr. G. Dalldorf of the Division of Laboratories and Research of the New York State Department of Health, antisera were received for the viruses which he has designated as *types 1*, *2* and *3* of *group A*.

TABLE 4. DISTRIBUTION OF VIRUS TYPES

STATE	TYPE 1[1]	TYPE 2[1]	TYPE 4	TYPE 5
Ala.	1	5	18	9
Colo.			2	
Del.	1	6	3	
Fla.				7
Ga.		3	6	3
La.		3	1	1
Okla.		1	2	
S. D.			1	
Tenn.	1	5	6	1
Tex.				1
Total no. types[2]	3	23	39	21

[1] *Types 1* and *2*: Sickles and Dalldorf.
[2] Each number represents an individual case.

By means of both the neutralization and the complement-fixation tests (8), it was found that certain of the strains isolated in this laboratory fell into *types 1* and *2* but none so far into *type 3*. In addition 2 other immunologically distinct viruses have been found that are tentatively called *types 4* and *5*. Table 4 shows the geographical distribution according to states of the 86 strains that have been typed. So far, *type 4* has been found most frequently while *types 2* and *5* are next in prevalence. Although there was a tendency for a certain type to predominate in one area or in a particular outbreak, yet often one or more other types might be present also. *Type 5* seems to be prevalent in Florida and in Alabama.

All 4 of the strains were serologically distinct but had certain properties in common: *1*) They could be propagated only in suckling mice or hamsters, did not infect 14- to 21-day old mice, embryonated eggs, young chicks, baby guinea pigs, rabbits or monkeys, including one 4 days old; *2*) they all passed through a Seitz filter and *3*) produced myositis but no neurological lesions in suckling mice. Virus could be found in the blood stream, the brain, cord, lungs, liver, spleen, kidneys and intestinal contents of infected mice and was present in high concentration in the skeletal muscles. Likewise the baby mice could be infected by various routes, intracerebrally, intraperitoneally, intramuscularly, subcutaneously and intranasally.

ISOLATION OF TYPE 4 VIRUS FROM NORMAL MICE

Early in this study a question arose as to whether or not one or more of the Coxsackie strains might not be latent in normal mice. In May 1949, the filtered muscles of 4 normal 3-day old mice were inoculated into a group of 5-day old mice. In 4 days the animals became paralyzed and a second passage of filtered muscles to normal suckling mice resulted in establishment of a virus that was serologically different from the other 2 strains on hand and was later called *type 4*.

On reviewing the records, it was noticed that 2 Coxsackie strains that were later proven to be *type 4* were inoculated on the same day as the no. 1 normal mouse strain and it also happened that at least 7 strains of virus that later proved to be *type 4* had been isolated in the laboratory from human material during the preceding 2 months. This was unknown at that time.

The question now arose as to the origin of this *type 4* strain. Was it a latent mouse virus inactivated by inoculation or was it originally of human origin but so easily passed in the laboratory that it could be picked up in normal mice on serial passage?

From the facts that antibodies for *type 4* strain were found in human sera, that this virus could be obtained more than once from the same human tissues and could be found in mouth washings at weekly intervals, it seemed probable that the *type 4* strain might have a human origin but could be acquired by mice in the laboratory. Although no virus was recovered from the tissues of 3 pools of suckling mice tested at that time, yet from later experiments it seems highly prob-

able that accidental infection can occur in the laboratory. The same *type 4* virus has been isolated on 2 other occasions from the filtered muscles of normal mice that had been inoculated with filtered muscles of other normal mice.

ANALYSIS OF VIRUS ISOLATIONS

Because of the above findings and because so many viruses were being obtained, the isolations were analyzed according to the number of transfers made in suckling mice before typical muscle paralysis was observed. Figure 1 shows the number of isolations of each virus type obtained after 1, 2, 3 or 4 passages, respectively, in suckling mice. Of 126 isolations, only 28 (22.2%) viruses gave typical symptoms in mice after the first inoculations, 56 (44.4%) were definitely positive after a second transfer, 37 (29.2%) strains were obtained after a third passage, while it took 4 transfers in mice to recover 5 (3.9%) of the strains. In each instance such symptoms as lack of growth, slight muscle weakness and ataxia were observed in the mice that warranted the transfer of the muscle filtrates to more animals.

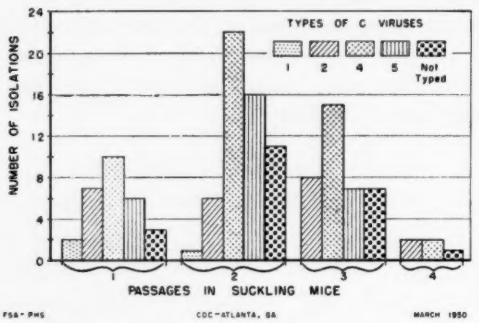

FIG. 1. PASSAGES IN MICE before muscle paralysis occurred.

Besides these 126 isolations of the Coxsackie viruses, 200 other human specimens were inoculated into baby mice without recovery of a virus. One hundred and forty-six of the specimens were passed only once, while 44 were passed twice in mice, and 10 were given 3 passages without virus isolation. Therefore, out of 326 attempted isolations, 126 (38.6%) were positive for the Coxsackie viruses. It seems probable that some of these recoveries were not obtained from the human cases, but resulted from accidental transfer in the laboratory. The authentic strains are most likely those that show definite muscle paralysis on the first passage, although many obtained after 2 mouse transfers may also be considered.

SEROLOGICAL TESTS

Both neutralization and complement-fixation tests have been done on groups of human sera, many of them taken during the acute and convalescent stages (8). As shown in table 5, of 55 paired sera only 15 (27.2%) showed evidence of a rise in neutralizing antibodies between the early and late specimens. Twenty-six (27.1%) of 95 sera tested neutralized more than one virus, although not all the specimens were tried against each of the 5 strains. The sera of 26 (72.2%) out of 36 patients from whom a virus was iso-

TABLE 5. RESULTS OF NEUTRALIZATION TESTS AGAINST COXSACKIE VIRUSES

A

Sera	TESTS AGAINST VIRUS TYPES							
	1		*2*		*4*		*5*	
	No.	Positive	No.	Positive	No.	Positive	No.	Positive
Patients	43	19 (44.1%)	59	53 (89.8%)	29	19 (65.4%)	19	14 (73.6%)
Normal			4	4 (100%)	14	10 (71.4%)		

B

Total number of paired sera tested	55
Cases with negative acute phase sera but positive convalescent	15
Sera positive to 2 viruses	26
Sera positive to the patient's virus	26
Sera negative to the patient's virus	6
Sera positive to a heterologous virus	4

lated contained antibodies for the homologous type of Coxsackie virus, while 6 sera failed to be neutralized by the homologous strain and 4 were positive to a different type.

The results of the neutralization tests against each of the 4 strains of virus are given also in table 5. Of 17 normal sera tested against *types 2* and *4*, only 4 were negative against the viruses employed.

DISCUSSION

Although 126 isolations of the Coxsackie viruses have been made from tissues or secretions of 105 individuals, yet from the fact that the most prevalent type (*type 4*) has been recovered from filtered muscles of normal suckling mice, it seems quite likely that many of these isolations were due to dissemination of the virus in the laboratory. This is to be considered particularly probable, because on several occasions 2 different types of Coxsackie virus were obtained either from the same or from different specimens from one individual. These irregular findings have occurred at least 4 times.

In this study it has been of special interest to have recovered the Coxsackie viruses (*types 2*, *4* and *5*) from the tissues or secretions of 12 different fatal cases, 6 of which were diagnosed as poliomyelitis. Poliomyelitis virus was isolated from the spinal cord of one of these patients and also from the feces of 2 recovered cases. Because of the lack of uniform symptoms, however, it is still a matter of conjecture whether or not the Coxsackie viruses were responsible for the fatalities.

The isolation of the Coxsackie viruses from the blood stream of human cases is likewise of significance, not only because it suggests a simple method of recovering virus, but also because the blood is thus a potential source of virus for blood sucking vectors. Melnick *et al.* (10) have reported previously on the recovery of the Coxsackie virus from flies taken in nature. However, because of the frequency of isolating the virus from the oral passages, the evidence at present seems to indicate a person-to-person transmission.

Certain strains of the Coxsackie viruses seem to predominate in particular outbreaks and in certain areas. Where the infection seems to be mixed, the possibility of accidental contamination is to be considered. If one adheres to the following criteria, however, one should be reasonably certain that the virus has come from the patient; *1*) typical muscle paralysis in the first mice inoculated with the original specimen, *2*) ability to recover the same strain of virus more than once from the same material and *3*) presence of antibodies in the patient's serum to the same strain of virus isolated from the patient, preferably with evidence of a rise in titer.

SUMMARY

Strains of the Coxsackie group of viruses have been isolated from the tissues or secretions of 105 patients from 10 different states with multiple isolations from different kinds of specimens from 6 separate individuals. The widespread prevalence of this group of viruses is thus made apparent. Four distinct serological types have been obtained. Two of them correspond to *types 1* and *2* of Dalldorf, the other 2, tentatively called *types 4* and *5*, are antigenically different, although giving the same clinical and pathological pictures in mice. These viruses have been recovered from human blood, serum, brain, cord, mouth or nasopharyngeal washings, saliva, sputum, nasal swabs, mouth vesicles, and feces, with a predominance of *types 4*, *2* and *5*, in order of frequency. The viruses have been found in material from 12 fatal cases from 7 different states and have been recovered many times from the blood stream.

Neutralizing antibodies for the Coxsackie viruses have been found in the sera of both patients and normal individuals. Of 55 paired sera 15 (27.2%) showed an increase in antibody content between the acute and convalescent specimens, while 26 sera neutralized more than one type of virus. Antibodies for the homologous strain of virus were found in 26 (72.2%) of 36 sera of patients from whom a virus had been isolated. One of the strains of Coxsackie virus has been recovered 3 times from muscle filtrates of normal suckling mice inoculated with other normal mouse muscles and there is strong evidence to show that these viruses may spread accidentally in the laboratory, thus accounting for some of the isolations. To be assured that a particular virus has etiological significance, it should *1*) produce typical muscle paralysis in suckling mice on the first inoculation, *2*) should be recovered a second time from the same material and *3*) should be neutralized by the patient's serum.

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DIVERSE MANIFESTATIONS OF INFECTION WITH A STRAIN OF COXSACKIE VIRUS

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SINCE THE ORIGINAL ISOLATION by Dalldorf and Sickles (1) of new viruses pathogenic for suckling mice, the properties of the Coxsackie viruses have been extensively investigated. However, studies of the disease produced with these viruses in man have been few, and largely limited to consideration of patients suspected of poliomyelitis. It is already evident that infection of the human with Coxsackie viruses may result in paralytic disease (2), aseptic meningitis (3), epidemic pleurodynia (4) or undifferentiated fever (4). The present report is concerned with a comprehensive study of the varied manifestations of infection with a strain of Coxsackie virus in a small, circumscribed outbreak unassociated with paralysis or meningitis.

In early August 1948, 5 of 6 physicians resident at the Hospital of The Rockefeller Institute and 2 non-resident physicians closely associated with the Hospital fell victim within a 2-day period to an acute febrile illness. Sporadic instances of illness also occurred concomitantly in other members of The Rockefeller Institute staff unassociated with the Hospital. Efforts to demonstrate serologic evidence of infection with the viruses of influenza A, influenza B, and Newcastle disease were unsuccessful in the early study of this influenza-like epidemic. Infectious mononucleosis, suspected because of the presence of atypical lymphocytes in blood smears, was excluded by the absence of significant concentrations of sheep red blood cell agglutinins in convalescent sera of patients. Attempts to recover a virus from blood and throat washings by inoculation of chick embryos and 3-week old Swiss mice were fruitless.

The report, in 1949, by Melnick, Shaw and Curnen (3) of the isolation in suckling mice of a virus from aseptic meningitis patients in New England in the summer of 1948, prompted reinvestigation of The Rockefeller Institute outbreak because of its temporal coincidence with the New England epidemic. Neutralization tests employing the Connecticut strain (Conn. 5) of virus (kindly supplied by Dr. J. L. Melnick) against the paired sera of 7 patients promptly defined the etiology of The Rockefeller Institute illnesses. Because these patients had presented no clinical evidences of paralysis or meningitis, as described in earlier reports of Coxsackie virus infection, it seemed of importance to review the comprehensive clinical and laboratory data assembled on the 5 hospitalized patients in order to aid in the delineation of this newly recognized disease.

CLINICAL MANIFESTATIONS

Within the same 12-hour period, 6 of the 7 patients studied experienced the onset of fever. In all but one patient insidious prodromata of insomnia, malaise and migratory aches of low intensity had occurred during the preceding 24 to 48 hours.

Certain features of the disease encountered in 3 or more of the 5 hospitalized patients are listed in table 1. The major complaint in all patients was pain of aching character, usually described as 'muscular.' Pain involved the neck, eyes, back, head, legs and shoulders with greatest frequency. Restlessness was also described by all patients, and occurred chiefly in the afebrile, prodromal period. It is notable that 4 patients complained of mild pharyngeal soreness; in 3 this symptom appeared 48 hours after onset of fever. Fever and muscle tenderness were physical signs common to all patients. In 4 patients fever lasted less than 24 hours. The fifth patient suffered a febrile relapse on the sixth day of illness unassociated with the presence of bacterial pathogens in blood or throat cultures.

Except for the non-specific common complaints of pain and fever, there was little clinical indication that the patients studied were victims of the same disease. The diverse clinical manifestations of infection are contrasted in table 2. It is apparent that such widely divergent clinical entities as infectious mononucleosis, acute appendicitis, sinusitis, pleurodynia and influenza

were simulated by Coxsackie virus infection. Despite the brevity and benignity of the symptoms, the intensity of the abdominal and 'sinus' pain encountered was sufficient to instigate surgical consultation, while the severity of the chest pain in *patient H. A.* suggested acute pleuritic or pericardial disease, the latter belied by a normal electrocardiogram.

Physical signs of disease were few. Slight pharyngeal injection and posterior pharyngeal lymphoid hyperplasia were noted in 3 patients. Generalized lymphadenopathy was not detected, but tender submaxillary lymphadenopathy was found in patients with pharyngeal soreness, and in one patient a small, tender axillary node was discovered. Splenomegaly of slight degree was discerned in 2 patients, one of whom complained of tenderness of palpation in the right hypochondrium and suprapubic region. The latter complaint was not associated with urinary tract signs or symptoms. Neurological examination in all patients was negative although *patient H. E.* remarked transient paresthesia in the right axilla.

Table 1. Common characteristics of disease

ONSET	GRADUAL
Common complaints	Pain (5)[1]: neck, eyes, back, head, legs, shoulders Restlessness (5) Mild sore throat (4) Nasal congestion (3)
Common signs	Fever (5): av. = 102.4 (R) Muscle tenderness (5)
Average fever duration	30 hours
Complications	None
Convalescence	Protracted asthenia

[1] Number of patients.

Despite the promptness of defervescence, convalescence in all patients was protracted, symptoms of transient aches, lassitude, generalized weakness and easy fatigability persisting for 1 to 5 weeks. During the ensuing 22 months no sequelae of infection have become apparent.

LABORATORY DATA

Routine urine analyses were negative, and none of the common pathogens was isolated from throat or blood cultures. Hematologic findings of interest are summarized in table 3. Unequivocal leukopenia was evident in 2, and low normal total leukocyte counts were observed in 2 other patients. Relative lymphocytosis was a feature of differential leukocyte counts made during the acute illness and also one month later. Large, atypical, young lymphocytes with deeply basophilic cytoplasm and lobulated nuclei were noted in 2 acute phase smears, and in the 4 convalescent phase smears studied. Eosinophilia occurrred in 2 patients in early illness.

Table 2. Diverse clinical manifestations of infection

PATIENT	CHIEF SYMPTOMS	CHIEF PHYSICAL SIGNS	ILLNESS SIMULATED	NEUT. ANTIBODY ACUTE/CONV.	VIRUS ISOLATION
E.K.	Headache	Splenomegaly	Infectious mononucleosis	<4/600	0
H.K.	Abdominal pain	Abdominal tenderness	Appendicitis	<10/62	+
H.E.	Backache Nasal obstruction	Sinus tenderness Nasal obstruction	Sinusitis	<10/390	0
H.A.	Chest pain	Splinting of chest	Pleurodynia	<10/256	0
C.S.	Chest pain Ocular myalgia Cough	Pain on eye movement	Influenza	<10/427	0

DIAGNOSTIC AND IMMUNOLOGIC STUDIES

Confronted with the problem of re-investigating, 14 months after its occurrence, an epidemic from which no etiologic agent had been recovered, the factor of geographical propinquity dictated the choice of the Conn. 5 strain of Coxsackie virus for trial neutralization tests with patients' sera. Tests were performed in 1- to 2-day-old Rockefeller Institute white mice by the intraperitoneal injection of 0.03 cc. of virus-serum mixtures containing 50 median effective doses (using death and paralysis as the end point) of virus in the form of saline suspensions of eviscerated suckling mouse carcasses. Preliminary tests in which 1:10 (final) dilutions of sera were used demonstrated no neutralizing antibody in any of

the 7 acute phase specimens, and complete protection by all convalescent sera.

Attempts to recover virus from the throat washings and blood of patients resulted in the isolation, on 2 occasions, of virus from the throat washing of *patient H. K.* This throat washing, taken in phosphate buffered saline (pH 7.2) had

TABLE 3. COMPARATIVE LEUKOCYTE COUNTS

PATIENT	TOTAL LEUKOCYTE COUNTS (ACUTE)	DIFFERENTIAL LEUKOCYTE COUNTS[1]									
		Acute					Convalescent				
		P	L	A.L.	E	M	P	L	A.L.	E	M
E.K.	2400	14	58	16	2	10	32	34	32		2
H.K.	11,200	52	12	22	8	3					
H.E.	3400	51	38		3	8	32	47	18	3	
H.A.	6600	55	38			7	50	20	20	4	6
C.S.	5400	34	32		30	4	35	21	37	6	1

[1] P = polymorphonuclear leukocyte. L = lymphocyte. A.L. = atypical lymphocyte. E = eosinophile. M. = monocyte.

been stored in a sealed glass ampoule for 14 months in a CO_2 cabinet prior to intraperitoneal injection into a litter of one-day-old mice. Five days later, tremors, ataxia and generalized weakness were noted, and all mice were killed for passage. On second passage, a 20 per cent broth suspension of extremities and brain tissue, injected intracerebrally and intraperitoneally, initiated paralysis as early as the third day. Third passage by the intraperitoneal route alone resulted in death or paralysis by the 5th day of all 16 mice inoculated. This virus (HK) was neutralized by specific immune serum against the Conn. 5 strain, while complement fixation occurred in the presence of HK antiserum and the Conn. 5 virus. (This and complement-fixation reactions subsequently mentioned were generously performed by Dr. Jordi Casals.)

Titration of neutralizing antibody in serum specimens was effected by mixing serial 4-fold dilutions of serum previously heated at 56°C. for 30 minutes with a constant quantity of virus (i.e. 50 median effective doses). This method of titration was chosen because it should provide accurate measurement of antibody concentration in sera with high neutralizing capacity. Studies by Horsfall of the precision of neutralizing antibody measurements with influenza A virus in the mouse (5, 6), and by Horsfall and Curnen of PVM neutralizing antibodies in the same species (7) have demonstrated with both viruses an identical linear relationship between the serum dilution end points and the quantity of virus employed. Morgan (8) has shown the same relationship using still another virus (W.E.E.) in the mouse inoculated by either the intraperitoneal or intracerebral route. In these systems the quantity of virus may be varied as much as 7-fold without causing a significant (i.e. 4-fold) change in the neutralization titer of a serum.

TABLE 4. NEUTRALIZING ANTIBODY TITRATIONS

PATIENT	VIRUS	TITER OF ACUTE SERUM 1–3 days	TITER OF CONVALESCENT AND LATE SERA				
			½ mo.	1–2 mo.	8 mo.	14 mo.	20–21 mo.
E.K.	Conn. 5	0[1]	>4096[2]	600	117	144	112
	HK		2560	410	115	64	131
H.K.	Conn. 5	0		62			32
	HK	0		132			16
H.E.	Conn. 5	0		390			32
H.A.	Conn. 5	0	256	256			128
C.S.	Conn. 5	0		427			64
			Complement-Fixing Antibody Titers				
E.K.	Conn. 5	0		8	4	2	2
H.A.	Conn. 5	0		2			

[1] No antibodies demonstrable in 1:10 dilution of serum. [2] Reciprocal of serum dilution.

In table 4 the neutralizing antibody titers of the 5 hospitalized patients may be compared. In every instance antibody was undetectable in acute phase serum, but manifest in high titer in convalescence. In 2 instances (*patients E.K.* and *H.A.*) when serum was obtained less than 2 weeks after onset of illness, very high antibody concentrations were already evident. High concentrations of antibody were still present in the sera of

all patients nearly 2 years after infection. Serial antibody titrations in *patient E. K.*, using both the Conn. 5 virus and the HK virus homologous to the epidemic, reaffirmed the antigenic similarity of the 2 strains, and provide an indication of the slow rate of decline of neutralizing antibody levels. Concomitant serial determinations of complement-fixing antibody are also recorded in table 4.

Studies of the sera of 2 laboratory workers heavily exposed to infected litters and tissue suspensions were made 4 and 7 months, respectively, after initial exposure. No neutralizing antibodies were manifest in the serum of the worker exposed for the longer period. However, serum drawn from the other worker 4 months after his original exposure to Conn. 5 virus was found to contain neutralizing antibody not initially present. During the period of exposure this person had experienced no clinical illness.

This study of an outbreak of Coxsackie virus infection has adduced certain facts worthy of emphasis: Coxsackie viruses, currently identified with poliomyelitis-like illness, may produce disease with widely divergent manifestations unsuggestive of poliomyelitis. Convalescence may be slow, and abnormal differential leukocyte patterns may persist for 4 to 5 weeks. Neutralizing antibody response is prompt, maximum serum titers appearing 2 weeks or less after the onset of illness, and high titers persist at least 21 months. Inapparent infection may occur and lead to a specific antibody response. The stability of the virus is indicated by its repeated recovery from a throat washing stored at −70°C. for 14 months.

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DIFFERENTIATION OF IMMUNOLOGICAL TYPES AMONG THE COXSACKIE VIRUSES[1]

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SOON AFTER THE DISCOVERY of the Coxsackie group of viruses, it was clear that they existed in multiple immunological types (1, 2). In fact, in view of the differences in tropisms for certain murine tissues which exist among the types (3, 4), it is not unlikely that different viruses may now be included in this group. For the present, however, viruses whose capacity to produce overt disease in experimental animals is limited to infant mice and hamsters are being placed in the Coxsackie family. It is recognized that in mice some types (*group A*) produce a disease which seems to involve only the skeletal muscles, whereas other types (*group B*) also involve additional tissues, notably the brain. In any case, we shall deal here with methods for classifying strains of what are now considered to be the Coxsackie viruses into distinct immunological types.

The classical methods of immunology have been employed with certain modifications which have had to be adapted to these viruses and their infant hosts. The methods include: *1*) cross protection tests in infant mice born of vaccinated mothers, *2*) production of pharyngeal and intestinal carriers of virus in chimpanzees following its oral administration, and a study of homologous and heterologous immunity in these animals, *3*) cross neutralization tests, and *4*) cross complement-fixation tests.

CROSS PROTECTION TESTS IN INFANT MICE

The susceptibility of mice to Coxsackie viruses is short-lived, and in a matter of a few days after birth they become naturally resistant to the disease. Consequently, cross protection tests in which challenge virus is inoculated into vaccinated mice cannot be performed, because the time required for an expected immunity to develop would place the mice out of their susceptible period. But by challenging infant mice within 48 hours of birth from mothers vaccinated with different strains of active virus, we have been able to use this procedure as a typing method. The vaccinated females which were bred had neutralizing antibody titers of about 1:1000 against 100 ID_{50} of virus and complement-fixing titers of about 1:64 against 8 units of antigen. It was found that homologous immunity was present whether or not the baby mice were nursed by their own or by foster mothers following the challenge inoculation of 100 ID_{50} doses of virus. However, in practically all instances the infant mice received at least one feeding of colostrum from their own mother before the inoculation of virus. The results are shown in table 1.

TABLE 1. MATERNAL TRANSFER OF TYPE SPECIFIC IMMUNITY

CHALLENGE VIRUS	BABIES BORN OF MOTHERS VACCINATED WITH			
	Conn.-5	Ohio-1	Texas-1	High Point
Conn.-5	+	−	−	−
JLM	+		−	−
NL		−	−	−
Ohio-1	−	+	−	−
Texas-1	−	−	+	+
High Point	−	−	+	+
NH Fly 1943				+
Easton-2	−	−	−	−
NY-5		−	−	

+ indicates mice resistant to challenge inoculation.

− indicates mice susceptible to challenge inoculation.

The immunity was type specific and was completely absent when mice were challenged with heterotypic viruses. Strains which fall into the same antigenic type by other tests (neutralization and complement fixation) also fall into the same type in these cross protection tests. Thus mice

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

vaccinated with the High Point or Texas-1 strain had babies immune to each of these strains and to the related NH Fly 1943 strain. Also when 2 strains (*JLM*, *NL*) related to Conn. 5 were used for challenge, only mice from Conn.-vaccinated mothers were protected. And when 2 strains (Easton-2 and NY-5) related to Dalldorf's *type 1* were used, none of the offspring from mothers vaccinated with any of the 4 strains was protected.

HOMOLOGOUS AND HETEROLOGOUS IMMUNITY IN CHIMPANZEES

In following the immune response in chimpanzees which have no obvious signs of illness following oral infection with Coxsackie virus, criteria other than clinical illness must be used. The measure of infection is based on the development of a pharyngeal and intestinal carrier state (with viremia) and the accompanying appearance of neutralizing antibodies in the serum. The measure of immunity is based on whether or not the carrier state can be again produced by a challenge dose of virus. Similar studies in chimpanzees infected with poliomyelitis virus have recently been reported (5, 6).

The pattern of the results obtained (7) is shown in figure 1. If the Conn.-5 strain is fed to a chimpanzee, the virus will appear in the blood for a few days following the feeding. Virus may be recovered from the throat generally starting a few days after the exposure and continuing for 3 to 6 days. Some, if not the bulk, of the virus fed is excreted within 3 to 5 days, and this is followed by new virus, 'manufactured' by the animal, for a period of 2 to 4 weeks. Antibodies to the virus appear within 2 weeks and maintain their titer for many months.

Several weeks later, if the same strain is re-fed to such a chimpanzee, then the virus may be found in the stools for a period of about 5 days, having passively traveled through the alimentary tract. No true carrier state occurs, and it has not been possible to isolate virus from the blood or throat. However, if the Texas (heterotypic) strain of virus is now fed, then the chimpanzee appears to respond in the same manner as it did when the Conn. virus was first fed, and a new antibody (Texas type) appears in the blood. Such a chimpanzee is now immune to both types, but may still respond like a new animal when fed a third type (Ohio).

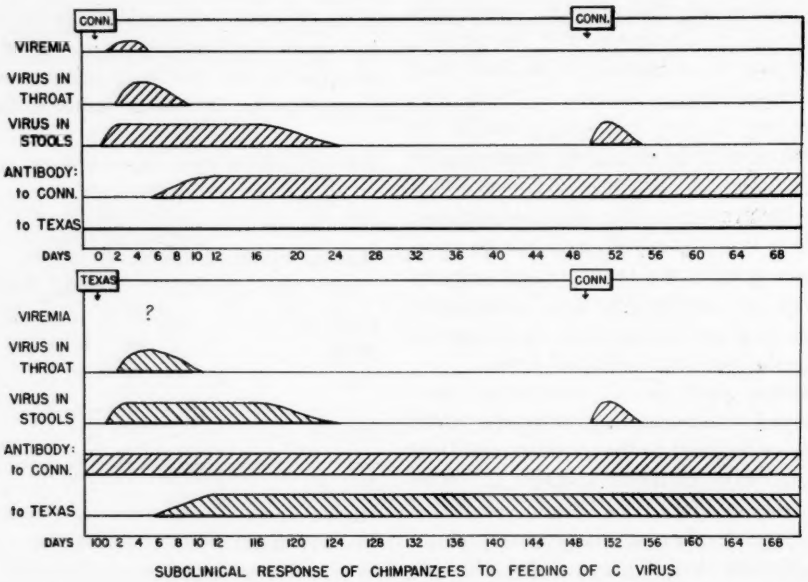
CONN.
CONN.
VIREMIA
VIRUS IN THROAT
VIRUS IN STOOLS
ANTIBODY: to CONN.
to TEXAS
DAYS 0 2 4 6 8 10 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68
TEXAS
CONN.
?
VIREMIA
VIRUS IN THROAT
VIRUS IN STOOLS
ANTIBODY: to CONN.
to TEXAS
DAYS 100 2 4 6 8 10 12 116 120 124 128 132 136 140 144 148 152 156 160 164 168
SUBCLINICAL RESPONSE OF CHIMPANZEES TO FEEDING OF C VIRUS

Fig. 1. DEMONSTRATION OF HOMOTYPIC IMMUNITY and heterotypic susceptibility in the chimpanzee.

With this method we have found that the Conn.-5, Texas-1 and Ohio-1 strains each belong to a distinct immunological type and that the High Point strain is related to the Texas type.

CROSS NEUTRALIZATION TESTS

Type-specific neutralizing antibody is one of the most widely used reagents for identifying a virus, and for the Coxsackie group of viruses the

neutralization test works admirably if adequate numbers of infant mice are available for the performance of the tests. For diagnostic purposes, using paired sera, or for other quantitative antibody measurements, results are most satisfactory when a fixed quantity of virus is used with varying dilutions of serum. For typing of strains, however, we prefer to use a constant amount of serum (usually undilute or 1:5 mouse or monkey immune serum) and determine the titer of the strain in the presence of this serum. In conformity with the procedure used for other viruses, the neutralization index is the difference between the titer of the virus in the presence of the immune serum and that in the presence of normal serum. Pre-heating of the serum at 56° C. for 30 minutes has no measurable effect on the content of antibodies.

TABLE 2. CROSS NEUTRALIZATION OF STRAINS OF COXSACKIE VIRUS

VIRUS	CONTROL ID_{50}	IMMUME SERUM: LOG OF NEUTRALIZATION INDEX							
		Conn.	Ohio	Texas	Hi.Pt.	Easton	D-*1*	D-*2*	D-*3*
Conn.-5	$10^{-5.7}$	>4.7	0	0	0	0	0	0	0
W-S, NC	$10^{-4.3}$	>3.3	0	0	0	0	0	0	0
Ohio-1	$10^{-5.2}$	0	>4.2	0	0	0	0	0	0
Texas-1	$10^{-7.0}$	0	0	6.0	5.0	0	0	0	0
Hi. Pt.	$10^{-5.5}$	0	0	4.6	5.0	0	0	0	0
NH '43	$10^{-7.5}$	0	0	6.0	6.0	0	0	0	0
Easton 2	$10^{-6.0}$	0	0	0	0	5	5	0	0
D-*1*	$10^{-6.0}$	0	0	0	0	5	5	0	0
D-*2*	10^{-5}	0	0	0	0	0	0	>4	0
D-*3*	10^{-5}	0	0	0	0	0	0	0	>4
Nancy	10^{-6}	0	0	0	0	0	0	0	0

The results of table 2 indicate that we can now recognize at least 7 antigenic types (7). Of these, 2—Conn.-5 and Ohio—belong to *group B* and 5, to *group A*. It is noteworthy that strains belonging to one type may be widespread in their occurrence. For example, the *group A* Texas type, named from the fact that it was first isolated from flies trapped in Texas in 1948, has been recovered *a*) from human stools collected that year and the next in Texas, *b*) from sewage collected in North Carolina in 1948, and *c*) from flies collected in New Haven in 1943 and stored on dry ice until 1950. Furthermore, antibodies to this type are widespread in the human population, occurring in sera collected in several areas in this country, in Europe and in northern Alaska. *Group B* types may also be widespread, as evidenced by the isolation of the Conn.-5 type in 1948 from patients in New Haven, Connecticut and Winston-Salem, North Carolina, and in the same year from sewage collected in Hartford, Connecticut. Antibodies to this type are also widespread, occurring in human sera collected on both the east and west coasts of the United States as well as in Denmark and Sweden. That the detection of these antibodies reflect prior infection with specific types of virus is suggested by the presence of Texas type antibodies and the absence of Conn. type antibodies in the sera of over 50 individuals, both young and old, living in northern Alaska (7).

Further information on the specificity of the neutralizing antibody response has been obtained from an extensive study of the occurrence and development of antibodies in 6 patients who contracted infections with one or another of the Coxsackie viruses while working with them in the laboratory (7–9). From each patient a virus was isolated during the acute phase of illness which was typed by following the reactions with specific hyperimmune animal sera (see bottom three rows of table 3). The data in table 3 also show that no patient before his illness had detectable antibodies to the strain subsequently isolated from him or to the prototype strain. But soon after illness, there developed simultaneously antibodies to the strain isolated from the patient and to the prototype strain to which it was related.

The reactions of the sera of the laboratory patients with strains not related to the one isolated from each patient are instructive. Only 2 of the 6 patients had an illness due to Texas type infection, yet the other 4 must have had a subclinical infection with this type. The results with the serial samples of blood taken from patient

JLM starting several months before his illness emphasize this point: on November 29, 1948, antibodies were absent to both the Conn. and Texas types. By February 21, 1949, antibodies to the Texas type had appeared in titer of 1:100 prototype. By May 26, such antibodies were present.

Concerning antibodies to the Ohio type, 2 patients had such antibodies before they became ill (to a heterologous type). Three of the remaining 4 persons did not develop Ohio-type antibodies during the period studied, whereas a fourth, *LMK*, did at the time of illness. The *LMK* strain proved to belong to the Ohio type.

It is noteworthy that this experience serves as an accidental but nevertheless clear example that

TABLE 3. ANTIBODY RESPONSE IN SIX PATIENTS INFECTED WHILE WORKING WITH COXSACKIE VIRUSES

PATIENT AND DATE OF ONSET	DATE OF SERUM	SERUM TITER OBTAINED AGAINST 100 ID_{50} OF VIRUS								
		Virus stain used in neutralization test								
		ESm_1[1]	*JLM*m_1	*NL*m_1	*LMK*m_1	*FL*m_1	*GJ*m_1	Conn.-5 m_6	Texas m_4	Ohio m_2
EWS	1/21/49	0						0	0	0
2/14/49	2/21/49	50						100	100	0
	3/25/49	250						100		0
JLM	11/29/48		0					0	0	100
5/16/49	2/21/49		0					0	100	10
	5/18/49		0					0	100	100
	5/26/49		250					100	100	1000
	6/12/49		1250					100	100	100
	10/20/49							100		100
NL	2/10/49			0				0	0	100
5/21/49	5/26/49			1250				100	100	100
	11/2/49							1000	100	100
LMK	3/25/49				0			10	10	0
10/20/49	5/24/49				0			10	1000	0
	10/28/49				100			1000	100	100
	12/27/49							100		1000
FL	6/15/49					0		0	0	0
7/16/49	7/25/49					100		0	1000	0
GJ	6/15/49						0	0	0	0
7/6/49	7/25/49						250	0	100	0
	Conn. mouse 10/20/49	>100	>100	>100	0	0	0	1000	0	0
Animal Immune	Texas mouse 10/22/49	0	0	0	0	>100	>100	0	1000	0
	Ohio Rh. 4332 8/11/49	0	0	0	>100	0	0	0	0	1000

[1] Subscripts after *m* indicate number of passages the virus had undergone in mice before it was used in the neutralization tests.

strains belonging to the Conn.-5, Ohio, and Texas types are capable of producing disease in man.

At the time of *JLM's* infection with the Conn. type, his family was infected with yet another type. His daughter, Nancy aged 8, was ill with 2 bouts of fever (maximum 103.5°), the first from May 14 to 16 and the second from May 27 to 28.

TABLE 4. FAMILY INFECTION WITH TWO TYPES OF COXSACKIE VIRUS

SERUM	SERUM TITER OBTAINED AGAINST 100 ID_{50} OF VIRUS					
	JLM m_1[1]	*Nancy*- m_1	*SM* m_1	Conn.-5 m_6	Texas m_1	Ohio m_2
JLM[2]						
2/21/49	0	0	0	0	100	10
5/18/49	0	0	0	0	100	100
5/26/49	250	1250	250	100	100	1000
6/12/49	1250	1250	250	100	100	100
Nancy[3]						
3/23/49	0	0	0	0	250	0
6/3/49	0	100	50	0	250	0
SM						
6/3/49	0	0	0	0	250	0
12/29/49	0	0	0	0	250	0
Conn.	>100	0	0	1000	0	0
Texas	0	0	0	0	1000	0
Ohio	0	0	0	0	0	1000
D-*1*	0	0	0	0	0	0
D-*2*		0	0	0	0	0
D-*3*		0	0	0	0	0

[1] Subscripts after *m* indicate number of passages the virus had undergone in mice before it was used in the neutralization tests.

[2] *JLM* had fever from 5/18 to 5/23.

[3] *Nancy* had fever from 5/14 to 5/16 and recurrence on 5/27 to 28.

Virus was isolated from fecal samples collected May 21 and June 5. Her strain belonged to a hitherto unknown type (7), shown by the data in table 4. Nancy's mother, *SM*, was a healthy carrier of this strain as evidenced by isolation of the virus from her stools collected on June 10. However, unlike Nancy, she did not develop neutralizing antibodies to her own virus, nor to the Conn. type which was the causative agent of her husband's illness. Nancy developed antibodies to her own virus, to her mother's virus, but not to her father's virus. Between May 18 and 26, her father developed antibodies not only to his virus and to the Conn. type to which it was related, but also to the type which infected the other 2 members of the family. The only reason for interpreting his illness as due to the Conn. type and not to Nancy's type was the isolation of a Conn. type virus during his illness.

COMPLEMENT-FIXATION TESTS

In determining the cross reactions between strains by the complement-fixation test, the plate method of Fulton and Dumbell (10) has been employed whereby complement and serum are varied with a constant amount of antigen. Table 5 shows the extent of the cross reactions between 7 strains of Coxsackie virus. The means for arriving at these figures are similar to those described for influenza virus (10) and will be described in detail for Coxsackie virus, but for the present it will suffice to say that '100' indicates a complete cross and '0' no crossing whatever. It is evident that the only extensive cross reaction occurs between strains High Point and Texas which together form a type immunologically distinct from the other 5 strains listed which in turn fall into 5 distinct types.

TABLE 5. CROSS REACTIONS OF COXSACKIE VIRUSES BY COMPLEMENT FIXATION

SERA	ANTIGENS						
	Texas-1	Hi. Pt.	Conn.-5	Ohio-1	D-*1*	D-*2*	D-*3*
Texas-1	100	122	0	0	0	0	0
Hi. Pt.	51	100	15	4	0	0	0
Conn.-5	0	10	100	0	0	0	0
Ohio-1	0	0	0	100	0	0	0
Dalldorf *1*	0	0	0	0	100	0	0
Dalldorf *2*	0	0	0	0	0	100	0
Dalldorf *3*	0	0	0	0	0	0	100

In preparing complement-fixing antigens for use with sera from heterologous animal species, antigens have been rendered specific for Coxsackie virus by treatment with protamine sulfate according to the method of Warren (11, 12). Table 6 shows the results of protamine treatment of mouse muscle antigen tested against immune sera of monkeys inoculated with mouse tissue. It

is seen that the nonspecific reactions of immune as well as of normal sera obtained with crude antigens (untreated) are eliminated when protamine treatment is employed.

We have occasionally failed to make satisfactory antigens for the low-titer types (Ohio, Conn., Dalldorf's *type 1*) and have resorted to concentration by ultracentrifugation (7, 12). Numerous tests have been performed supporting the logic of this procedure, and table 7 indicates

TABLE 6. SPECIFICITY OF PROTAMINE-TREATED ANTIGENS

MONKEY NO.	IMMUNE TO	STATE OF IMMUNIZATION	TITERS WITH ANTIGENS			
			Normal crude	Normal + protamine	Texas crude	Texas + protamine
4198	Texas	Pre	0[1]	0	0	0
		Post	4	0	64	16
3955	Texas	Pre	0	0	0	0
		Post	16	0	64	16
4151	Conn.	Pre	16	0	16	0
		Post	16	0	16	0

[1] 0 = <2.

TABLE 7. TITERS OF A SINGLE SERUM WITH ANTIGEN (EASTON-2) CONCENTRATED IN THE ULTRACENTRIFUGE

NO. OF TIMES ANTIGEN WAS CONCENTRATED	TITER OF SERUM
20	40
16	24
8	3
4	<2
With unconcentrated antigen	<2

typical results when the titer of a single serum is determined with various concentrations of a single batch of antigen.

The development of humoral complement-fixing antibodies in human infections with the Ohio type of Coxsackie virus has been studied (7, 12).[2] Table 8 indicates the serum titers obtained at various times after onset of illness in 6 patients. Concentrated antigen was employed in the tests, which were carried out in tubes using the 50 per cent hemolytic endpoint. Patient *LMK* had a proved laboratory infection with the Ohio type, and the same type had been isolated from *Red*, *Lin* and *Hux*. In the case of *Ric* and *Ris* no isolations were attempted, but it would appear from the complement-fixation results that *Ric* was infected whereas *Ris* was not.

TABLE 8. COMPLEMENT FIXATION IN HUMAN DISEASE USING CONVALESCENT SERA WITH OHIO TYPE OF COXSACKIE VIRUS

	TITER OF SERUM							
	Onset	2 wk.	1 mo.	3 mo.	4 mo.	7 mo.	1 yr.	2 yr.
Red	0[1]	7	32	112				8
Lin	0	3	4	56				6
Hux	0		4					2
Ric	0	0	0	192			96	
Ris	0		0	0				
LMK	0		0	24	30	12		

[1] 0 = <2.

TABLE 9. DIFFERENTIATION OF COXSACKIE VIRUSES BY FOUR METHODS

VIRUS TYPE	VIRUS NEUTRALIZATON	COMPLEMENT FIXATION	PROTECTION OF INFANT MICE BORN OF VACCINATED MOTHERS	IMMUNITY IN CHIMPANZEES TO REINFECTION
Conn.-5	X	X	X	X
Ohio-1	X	X	X	X
Texas-1	X	X	X	X
D-*1*	X	X		
D-*2*	X	X		
D-*3*	X	X		
Nancy	X			

X indicates that by the method employed, the type indicated has been proven different from each of the other types tested by that method. A blank space indicates not done.

The temporal pattern of complement-fixing antibody obtained with the Ohio type, however, is not necessarily the same as that experienced in infections with other types. Patients infected with the Easton, 1949, strain (belonging to Dalldorf *type 1*) seemed to develop such antibodies much earlier than those infected with the Ohio type.

SUMMARY

Immunological evidence has been presented that the Coxsackie group of viruses exists in multiple antigenic types. At least 7 types are

[2] All but one of these sera were obtained through the courtesy of Drs. Albert B. Sabin and Alex J. Steigman who made their collections in Ohio in 1947 (13). Isolations of Coxsackie virus from the stools of 3 of their patients were made (12).

now recognized. The methods for identifying these types have been reviewed in this paper. They are those of classical immunology with some modification to fit the virus-host systems involved and include: *1*) cross neutralization tests, *2*) cross complement-fixation tests, *3*) cross protection tests in infant mice born of vaccinated mothers and *4*) cross protection tests in chimpanzees with subclinical infections. It can be seen from table 9 that each method yields results in agreement with those obtained by each of the other methods employed.

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AMERICAN INSTITUTE OF NUTRITION

Symposium on Nutritional Appraisal of Human Subjects

Chairman: W. H. SEBRELL, JR.

EVALUATION OF THE SYMPTOMS AND SIGNS OF DEFICIENCY DISEASES[1]

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NUTRITION WORKERS ofttimes, and with some justification, accuse physicians generally of disregarding nutritional factors in the development of disease and of failing to accept some nutrition investigators' conclusions of widespread deficiency diseases within the population. Clinicians might reverse this indictment and accuse nutrition workers of ofttimes ignoring well-established clinical facts and of sometimes exhibiting a surprising ignorance of some fundamental medical concepts.

It is the writer's belief that the physician's sometime lack of enthusiasm for properly established nutritional claims has been attributable in part to the disappointment experienced when at other times nutritional claims were enthusiastically adopted, tested and found unsound. Accordingly, physicians have become cautious and even skeptical of all claims made by nutritionists. Because of such experiences, some clinicians are likely to regard nutrition workers as enthusiastic propagandists lacking in a knowledge of clinical medicine. It well behooves us as nutrition workers, therefore, to appraise critically the bases upon which we make claims, statements, policy recommendations and so forth. Otherwise we lose the confidence of other physicians and become ineffective.

Several examples could be cited to illustrate the above type of error. One such was the confusion of ordinary conjunctival thickening and pingueculae with xerosis conjunctivae or Bitot's spots. The claims were made (1) that these thickened areas in the conjunctivae were due to chronic vitamin A deficiency, that 99 per cent of a low income population exhibited this condition and therefore "had microscopic ocular lesions characteristic of avitaminosis A." In another study (2) it was stated that "according to the above criterion every subject carried unresolved degenerative changes resulting from vitamin A deficiency, most of them of a moderate to severe character."

The year following the appearance of these claims of a startlingly high incidence of avitaminosis A, an experienced ophthalmologist (3) pointed out that "The 'spots', or pingueculae, are subepithelial infiltrations, the epithelium over them being unaffected, a condition different from xerosis in which the changes are in the epithelium, and that such spots are not associated with low states of vitamin A, as proved by blood tests." Furthermore, this ophthalmologist pointed out that these states represent common presenile or senile alterations.

Grossly the Bitot spot is characteristically laterally placed, although they may be both laterally and medially. The lesion is greyish, dull, and has somewhat the appearance of meringue. This is in contrast to the more elevated, gelatinous appearance of pingueculae. Illustrations of a true Bitot's spot may be found in Berliner's monograph (4).

Epidemiologic investigations consisting of simultaneous dietary studies, measurement of vitamin A concentration in the blood plasma, and observations of the conjunctivae with the slit lamp were made in two separate areas in North

[1] Presented in the Symposium on Nutritional Appraisal of Human Subjects, Atlantic City, April 20, 1950.

Carolina (5, 6). The samples comprised approximately 1000 persons each. In neither study was a correlation found between the severity or incidence of the conjunctival changes and the level of vitamin A in either the diet or plasma. In a well-designed therapeutic study which tested the effect of the daily administration to nurses of 50,000 IU of vitamin A over a period of up to 2 years, Robertson and Morgan (7) found that upon examination with the slit lamp"... it was not possible to determine which nurses were receiving vitamin A and which were not... and that in none of the subjects with conjunctival elevations at the beginning did therapy cause the elevations to disappear. Therefore one would conclude that the conjunctival thickening is not caused by a deficiency of vitamin A." A similar lack of influence of vitamin A on the appearance of the thickenings was noted by Borsook and Wiehl (8).

In studies designed to produce avitaminosis A in human subjects, neither of these conjunctival lesions has been observed. The most extensive experimental study of vitamin A deprivation in man is that of the Vitamin A Sub-Committee of the Medical Research Council (9). Despite the production by dietary restriction of lowered carotene and vitamin A levels in the plasma and of abnormalities of dark adaptation, it was found that the various biomicroscopic changes observable initially in both the deficient and control groups did not vary with vitamin A intake. No Bitot spots developed.

The abundant evidence which has accumulated justifies the conclusion that the conjunctival changes which are commonly observed in our population are not related to avitaminosis A nor do they serve as useful measures of nutriture. Bitot spots are very infrequently seen in the population of the United States. When present they may at times indicate avitaminosis A but frequently are not evidence of vitamin A deficiency unless associated with other significant findings (10). Extravagant claims (11) concerning widespread avitaminosis A based upon the prevalence of these lesions in the population should be discarded.

What is the status of the skin lesions which have been said to signify avitaminosis A? First, one must be certain of the definition of these lesions, for confusion exists as to their characteristics. The workers who are most familiar with these lesions are investigators with experience in the Orient, India or Africa. The descriptions of the lesions indicate that they are hyperkeratotic papules occurring at the site of the hair follicles and initially, at least, over the extensor surfaces of the body. Because of the plugging of the sweat glands, the skin is excessively dry. The lesions are not to be confused with the congenital condition known as icthyosis... a confusion which I have observed repeatedly to occur when enthusiastic but inexperienced persons undertake their first nutrition surveys. Sometimes pityriasis rubra pilaris offers a differential problem (12). Furthermore, these lesions have been confused with a less common condition known as Darier's disease.

A more frequent and serious confusion seems to be that of hyperkeratosis follicularis with the common abnormality of skin texture known to dermatologists as keratosis pilaris. This condition would seem to be that which is sometimes referred to in the literature as 'permanent goose flesh'. It is not infrequent in children under 12 to 13 years of age regardless of their nutriture. These lesions tend to be less noticeable during the summer months when there is increased moisture of the skin and most prominent during the cold winter months when the moisture of the skin is decreased. I have observed these lesions in children who have had well-rounded diets plus supplementary vitamin A for the first 8 years of their lives and who exhibited satisfactory levels of vitmin A in the blood. Krause and Pierce (13) found no significant difference in the blood levels of children with and without the condition. These lesions are commonly seen in the United States. They cannot be taken as evidence of avitaminosis A. As a matter of fact, in a too-little-known paper which reports on 207 cases of avitaminosis A observed at Peiping Union Medical College, Frazier and Hu (14) pointed out that "The cutaneous eruption was characteristic of vitamin A deficiency only after the patient had attained sexual maturity. Before the age of puberty the only common abnormality of the skin (due to avitaminosis A-W.J.D.) was xerosis." These workers noted that of the 91 children under 15 years of age and diagnosed as having avitaminosis A, only 4 exhibited the keratotic lesions, and these 4 were all in the 14- to 15-year age group.

It appears, then, that lack of familiarity with the skin lesions of avitaminosis A has most likely led many workers in America erroneously to assign to this deficiency all of the 'nutmeg grater-like' skin lesions which they have observed. Most certainly, the frequency of occurrence of rough, dry skins with or without 'permanent goose flesh' cannot be used as the chief index of vitamin A nutriture. As a matter of fact, there is appearing

an increasing body of evidence relating skin lesions seemingly indistinguishable from the hyperkeratosis of which we have been speaking to a lack of other dietary essentials, especially the B vitamins, and even unsaturated fatty acids (15).

Recent studies show that clinically manifest avitaminosis A will be accompanied by serum levels of vitamin A of less than 50 IU per 100 cc. and that the earliest physiologic manifestation of this deficiency is the change in dark adaptation (9, 16). In my opinion, the only manner in which one may arrive at reasonable estimates of the incidence of vitamin A deficiency within a population is by the combined application of the clinical, biochemical and physiologic study coupled with observations on the therapeutic response of subjects judged deficient by these tests. Dietary studies may give confirmatory information, but they will chiefly be of value in the description of the food habits.

The question of avitaminosis A has been considered in some detail to serve as an example of the sort of critical appraisal of clinical methods which is desirable at this time.

Similarly, experience with the sign of so-called 'corneal vascularization' observable with the slit lamp as evidence for riboflavin deficiency has proved valueless for assessing the nutritional level population groups in this country. Although corneal vascularization does occur in ariboflavinosis (17), the occurrence of prominent vessels at the limbus, or indeed even of actual corneal invasion, is not correlated with the estimated riboflavin intake in the diet nor with other signs suggestive of riboflavin deficiency (5, 6, 18). Further, these conditions are not amenable to long-continued therapy with large quantities of riboflavin (8). Lastly, it may be added that vascularization of the cornea occurs in all types of corneal injury, and, at least in experimental animals, it may result from a great variety of dietary deficiencies (19–22).

It is to be concluded that this sign alone gives us no information as to the prevalence of ariboflavinosis within a population. In individual cases, however, it is reasonable to employ this sign as one of the several manifestations included in the syndrome of ariboflavinosis. This is to say that, as in all clinical diagnosis, we must not base our judgment upon the presence or absence of a single isolated non-specific sign.

Sebrell and Butler (23) demonstrated that fissuring at the angles of the mouth occurs in ariboflavinosis. This sign also occurs in deficiencies of iron (24, 25), niacin (26, 27) and vitamin A. It is often produced by illfitting dentures (28), in which instance it is termed 'pseudoariboflavinosis.' It may occur in chronic illnesses, especially arthritis, and not respond to vigorous nutritional therapy. Angular fissures are frequently observed to appear during periods of treatment with certain antibiotics. At times we have observed cases in which the lesions seem to be allergic in origin. Responses of angular fissures to pantothenic acid (29) as well as pyridoxin (30, 31) have been reported. It is apparent that interpretation of the significance of this sign must be made with considerable knowledge of the individual patient, and that a mere tabulation of the frequency with which it occurs in a population does not provide a reliable index of ariboflavinosis within that population.

The biomicroscopic appearance of the lingual papillae has been recommended (32) and used in some studies as an indicator of the niacin nutriture. The non-specificity of lingual changes is apparent from the variety of conditions which may result in glossitis. For example: Glossitis may be associated with iron deficiency anemia and respond to therapy with iron alone (24, 25). It is a frequently observed sign in sprue and responds to folic acid (33). The glossitis of pernicious anemia, a disease characteristic of the older age group, responds more satisfactorily or permanently to vitamin B_{12} than to folic acid (34). The deficiency glossitis of pellagra responds to niacin, but similar lesions may be amenable to therapy with riboflavin (17). Pantothenic acid (29) has been reported as efficacious in some instances.

Perhaps no other area of the body is subject to so great trauma as the mouth and tongue. The wearing of plates and the chewing of tobacco are frequent causes of glossitis and cheilosis (35). Post-menopausal atrophy of mucous membranes may be resistant to all forms of nutritional therapy. A legion of other non-nutritional lingual changes can be found in any good textbook of dermatology or of oral diagnosis. Among those most frequently referred to us for evaluation have been Moeller's glossitis, leucoplakia and lichen planus.

From the great variety of conditions which affect the lingual mucosa, only a few of which I have indicated here, it is apparent that biomicroscopic study of the condition of the tongue cannot serve to assess the niacin nutriture of a patient or a population.

It is well known that swollen, red, friable,

bleeding gums are characteristic of scurvy. It has been proposed (36) that changes in the gingivae serve as an index of vitamin C deficiency. Such an interpretation of these lesions would seem to receive support from studies on chronic ascorbic acid deficiency in the monkey (37) and from the investigations of Linghorne and others (38) on RCAF personnel. These investigations demonstrated that the recurrence of previously corrected gingivitis was retarded somewhat by the daily dietary intake of 75 mg. of ascorbic acid in comparison with subjects receiving 25 or 10 mg. of ascorbic acid. Without attempting to discuss all of the implications of this particular study, suffice it to note that gingivitis recurred (although more slowly) despite large intakes of ascorbic acid. Obviously there are causative factors other than vitamin C deficiency. Hence, gingivitis alone is not a valid criterion of ascorbic acid deficiency within the population. The gums, like other oral structures, are subject to many varieties of trauma and numerous gingival diseases are recognized by dentists and other oral diagnosticians. It is regretted that the guidance of those professionally trained persons with the best backgrounds in oral pathology has seldom been sought by nutrition investigators.

To summarize to this point: *1*) biomicroscopic examination is of very limited value in assessing nutritional level of individuals. It would seem to be useless in nutrition surveys as a generally applied procedure. Its usefulness is limited to the study either of individual cases of suspected ariboflavinosis or to observations of tissue changes which occur in controlled experimental investigations such as that of the Vitamin C Sub-Committee (39). *2*) A corollary of this conclusion is that estimates of the prevalence of nutritional deficiencies based upon this method should be discounted. *3*) Clinical signs of nutritional deficiency are not specific and for them to have usefulness in the detection of nutritional deficiencies within the population, they must be interpreted in relation to the medical (and dietary) history, other physical findings, laboratory studies, and the results of therapeutic tests. *4*) The nutrition investigator must recognize the limitations of his own training and background in clinical medicine, dentistry, ophthalmology and allied fields and work with competent individuals who are trained in these disciplines. Only by so doing will he make the greatest contribution to our total knowledge and avoid repetition of some of the expensive mistakes of the past.

Some of the signs widely used to 'judge nutritional status' appear to have been incorporated into surveys without clearly defined rationale. For example, it has repeatedly been suggested that alterations in the texture, pigment or amount of hair, particularly in children, may be due to malnutrition, and these qualitative alterations have been listed in tables of clinical findings useful in the evaluation of nutritional status (40, 41). Hair changes are indeed ofttimes noted in malnourished children (42, 43). By analogy to experience with animals, unkempt hair might be considered as a manifestation of illness due to deficiency disease. However, such changes in the human have not been linked with any specific nutrient, and from the analogy with animals, one might predict that if alterations in hair texture and color be indeed evidences of malnutrition they could arise from a great variety of deficiencies. Momentary reflection upon the problem indicates that numerous non-nutritional factors related to the hygiene of the scalp and hair influence hair texture.

The significance of some additional skin changes deserves mention. Keratotic lesions have been discussed. In Kwashiorkor a 'crazy pavement' dermatosis occurs (44). It would not be surprising if some uninitiated enthusiastic worker reports a series of patients with congenital ichthyosis as having 'subclinical Kwashiorkor'. Another skin lesion of some interest is petechiae. These small, usually perifollicular 'spots' are seen in early scurvy, where they are usually distributed over the dependent portions of the body or, in infants, in areas of trauma. Petechiae resulting from other causes, however, have similar distributions. For example, patients with idiopathic or orthostatic purpura exhibit petechiae of similar distribution to those seen in early adult scurvy despite high ascorbic acid levels in the serum. They do not respond to massive therapy with vitamin C or numerous other therapeutic agents, nutritional and non-nutritional. Petechiae are seen in toxic manifestations, allergic states and in blood dyscrasias. In some of the latter, they may be associated with gingivitis to present a more confusing diagnostic problem. Petechiae also occur in a number of infectious diseases and may be seen in limited numbers in older persons with senile atrophy of the skin. Obviously, evaluation of this sign must be made in relation to other findings.

The skin lesions of pellagra are usually symmetrically distributed over the exposed surfaces. The reason for this is that the pellagrin is photosensitive (45) and the skin changes are a mani-

festation of this. It is apparent, therefore, that very similar lesions will be produced in other photosensitive states, such as lupus erythematosus, in extensive vitiligo following exposure to the sun, ordinary sunburn, and so on.

So-called 'seborrheic dermatitis,' particularly of the face, nasolabial angle, and ears may result from a deficiency of riboflavin or niacin. However, indistinguishable lesions occur not infrequently in absence of deficiency states.

Neurologic manifestations of deficiency diseases include changes in the tendon reflexes and impairment of vibratory sense, both of which may result from peripheral neuritis. Peripheral neuritis may be a manifestation of thiamine deficiency, due to pernicious anemia, or have other etiologies. Under well-controlled conditions, repeated observations of these changes may provide some useful information regarding the development of deficiency diseases (46), especially if these observations be coupled with therapeutic testing. However, the multiplicity of factors affecting these two signs, together with the alterations which occur with advancing age, and the failure of the response to thiamine of the commonly occurring neurologic 'abnormalities' (47) demonstrate the restricted value of such observations for the determination of thiamine nutriture within a population.

I would be the last to deny that physical signs are an essential part of nutrition surveys when the observed signs are placed in proper relationship to known medical conditions which may result in these signs, along with well-conceived laboratory studies and dietary and therapeutic investigations. However, without such sound medical appraisal of the meaning of these signs, one should not make claims of the existence of widespread deficiency states based upon the physical findings alone.

Another word of caution concerning the evaluation and comparability of physical signs is necessary. This has to do with the subjectivity inherent in the clinical examination in nutrition —a problem well discussed by Bean (48) who noted that considerable differences may occur in the frequency with which examiners record the presence of a given physical finding within a population group. The interpretation or manner in which an examiner sees a condition or lesion depends, as Bean noted, very much upon the physician's own conditioning. For example, in studying cheilosis the clinicians who reported a very low incidence were familiar with the angular stomatitis or cheilosis which was frequently seen in severely malnourished individuals. They failed to record abnormalities of the lips which were noted by other observers.

In this relation, the following table from data from a study in which I have been interested is pertinent. Observers, whose initial appraisals were all made under one experienced worker, recorded their clinical judgment as to general nutritional status of portions of a population. The ratings were undernourished, adequately nourished and obese. The table presents the percentage judged to be undernourished or obese by a given observer. Great variation is seen in the judgments of these clinicians. Accordingly, we listed these data in approximately the order of

TABLE 1. PHYSICAL FINDINGS BY EXAMINER EXPRESSED AS PERCENTAGE OF TOTAL EXAMINATIONS BY THE EXAMINER

EXAMINER	GENERAL NUTRITURE	
	Under	Obese
R. C.	26.9	9.5
K. S.	24.3	12.6
E. W.	11.4	19.8
C. W.	8.4	16.9
W. Mc.	14.7	27.2
R. P.	3.1	13.8

the weight of the examiner. It is apparent that the clinician's concept of normal bore a relationship to his own weight; that is, the heavier physician was inclined to judge fewer people overweight and more people underweight than did the thinner physicians.

Obviously, there is a large and ofttimes immeasurable subjective influence in appraisals of physical signs. This must not be forgotten when one attempts to compare the frequency of occurrence of given lesions in one study with those reported for another. It would be of interest to have data which would test the constancy of the judgment of a single investigator over a period of years. I would predict that changes in the criteria employed by a given observer would be found.

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EVALUATION OF DIETARY SURVEY METHODS[1]

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THE ADVANCEMENT of the cause of nutrition depends upon a better understanding of body needs and of how these needs can be met through an appropriate food intake. Dietary surveys serve both of these objectives in a variety of ways. They serve to chart food consumption patterns which can be studied in relation to economic and educational status, cultural backgrounds and food habits, available food supplies and other factors. They provide the basis for more effective programs of nutrition education and for evaluating the results. On a national or regional basis, they furnish needed information for planning food production programs.

With respect to the topic of this symposium, they serve in the appraisal of the state of nutrition of individuals, families and other groups, through a comparison of the data obtained with some standard or yardstick of adequacy. They do not measure nutritional status directly. Rather they provide presumptive evidence, as well as yielding data which can assist in the interpretation of the results of more direct measures. In the past some workers have used the data from dietary surveys to draw conclusions regarding nutritional status, and particularly the extent of malnutrition, far more broadly than the methods used and their bases warranted. On the other hand, others who are particularly impressed by the limitations of the methods have expressed the view that surveys can have little value in the appraisal of nutritional health. Neither extreme viewpoint is justifiable. Surveys can contribute greatly if they are properly conducted and the results interpreted with a full recognition of the limitations involved.

Dietary surveys take many different forms, both with respect to the methods of obtaining data on food and nutrient intake, and to procedures for evaluating their meaning with reference to nutritional status. The various procedures have recently been comprehensively and critically reviewed in a section of the report of the Committee on Nutrition Surveys of the Food and Nutrition Board (1). This report should be carefully read by anyone who is planning work in the field. The present talk deals with certain aspects which are particularly pertinent in the use of dietary surveys as an aid in appraising nutritional status.

Whatever method of obtaining food intakes is used, some yardstick of adequacy is required to evaluate them. The reliability of the yardstick is thus of basic importance. At the present time there are certainly different concepts as to what constitutes malnutrition and, thus, as to what is an appropriate standard. In this country, assuming that the foods are translated into nutrients, it has become customary to use the Recommended Dietary Allowances. A direct comparison is made where the intakes are expressed as nutrients. Otherwise, servings or other measures of the foods themselves are compared with a recommended dietary pattern based on the Allowances. It is important, therefore, to recognize that these Allowances are higher than average needs, "to cover substantially all individual variations in the requirements of normal people." Parenthetically, the question arises as to what the word, normal, means. Not all individuals who fail to meet the Allowances can be considered to be malnourished. Some have failed to recognize this point in interpreting intake data.

Here also lies a limitation of food or nutrient intake data as measures of individual nutritional status. One does not know whether a particular individual needs the full amounts specified or whether substantially lesser intakes of some of the nutrients would suffice. As an illustration, in a small sample in Canada, Pett found that nearly 50 per cent of the adults were in calcium balance on an intake which was only half the 1945 Recommended Allowance and thus only 40 per cent of the present one (2). It may be considered that those 'normal' individuals who fully meet the Allowances or more are adequately nourished. Certainly, also, there is a level below which inadequacy can be assumed. In between lies a

[1] Presented in the Symposium on Nutritional Appraisal of Human Subjects, Atlantic City, April 20, 1950.

zone of uncertainty for the individual which further research may narrow. The Allowances provide no data for several nutrients now known to be essential. It is possible that a diet may meet all the stated Allowances adequately yet fail to provide all the nutritional requirements for health. The Allowances are based to a large extent on the findings obtained in biochemical studies and in medical inspections for signs and symptoms of deficiencies, which have been previously discussed in this symposium. Thus, the reliability of these findings must be considered in using the Allowances as a yardstick. The previous discussions have indicated that these biochemical and medical measures are still subject to much uncertainty as regards their significance. All of these considerations should rule out generalizations regarding the incidence of malnutrition from dietary data alone, but they do indicate the value of such data for the support and interpretation of other measures.

It is clear that individual data are needed in any appraisal of the status of the individual. Repeatedly it has been shown that the food intake of the family may not be distributed among its members in proportion to individual needs. Whatever method is used for the individual, the goal must be the actual food intake. Waste must be accounted for. It is agreed that the most accurate method of arriving at the actual nutrient intake is a laboratory analysis of duplicate meals, together with an analysis of the food not eaten, if any. The time, expense and special techniques involved limit the use of this method to a comparatively few individuals. It is not practicable for use in dietary surveys in the broad sense.

Thus, the reliability of other methods of assessing individual nutrient intakes is a question of special importance. The most commonly used procedure is the calculation of the nutrients consumed by use of food tables giving average composition. The question of the applicability of these calculated values as a measure of the intakes of individuals in specific dietary situations is an important one. Several recent studies, such as those reported and reviewed by Kaser and associates (3), Bransby and associates (4), and others, have led many to conclude that calculated values are not sufficiently reliable to assess the adequacy of the diet of an individual. As is to be expected, variations are greater in the case of certain vitamins than for other nutrients. Obviously, tables giving average composition cannot accurately reflect the actual nutrient intake from the food supply consumed in specific situations. Many factors cause the composition of staple foods to deviate from average values. Production, processing and storage variables are of particular importance for certain foods and in certain localities. Variable losses in home preparation must be expected, even though they are accounted for on an average basis.

These facts should cause us to consider how the influences of the variables in current tables of average composition can be minimized in future work, rather than arbitrarily to rule out calculated values for the individual. Advantage can frequently be taken of data for the composition of the local food supply at the season in question or of its nature as actually consumed, which may be more applicable than the average data. In a recent study by Hunter, *et al.* (5), in Canada, in which the food composition data selected were those considered most likely to apply to the conditions in question, reasonably good agreement was obtained, with a few exceptions, between calculated and analyzed values. The revised tables of composition, now in the process of publication by the Bureau of Human Nutrition and Home Economics, will certainly provide data which are more applicable to the foods now used and to specific situations as well.

Comparative studies of calculated and analyzed values should be continued with a fuller recognition of the possibilities of lessening the discrepancies previously reported. Not all of the differences should be blamed on the limitations of the average data on composition. Analyzed values may be subject to errors in measuring the food actually consumed and also to errors in carrying out the analytical methods. Unless the food intake data themselves represent reasonably well what was actually eaten by the individual, nutrient calculations not only are not worthwhile, but also may lead to very erroneous conclusions. Records consisting of weights or measurss of food eaten provide a more accurate basis than those expressed as servings.

The discussion thus far has dealt with procedures which give the greatest promise of reliability for appraising the nutritional status of the individual and for the support of and correlation with biochemical and physical findings obtained with the same subjects. Obviously it is impracticable to use them for any large segment of the population and for the broader purpose thus

served. The usefulness of family data for periodically assessing the food intakes, food habits and dietary trends of various segments of the population in relation to economic status, region, family size, food supplies available and other factors is well established. We are all aware of the large and important contributions which the Bureau of Human Nutrition and Home Economics are making along these lines.

With both family and individual food records the question as to whether or not the methods of collecting and evaluating the data can be shortened and still accomplish the purpose is of real importance in the interest of studying large groups. The calculation of nutrient intakes, food by food, is a very time-consuming process. The use of the so-called short method in which certain foods are grouped together greatly lessens the work involved. Several reports have indicated close agreement between certain short methods and the conventional long one. It has been shown, however, that short methods fall down where, in the diets under study, the foods making up a given group are consumed in quite a different proportion from that assumed in the grouping made. An intake of turnip greens as the sole representative of the green, leafy and yellow vegetable group should not be subjected to the same conversion factors as a weighed mixture of spinach, carrots, lettuce etc.

The recording of food intakes by servings is much simpler than by weight, and cooperation in obtaining records which can be considered reliable is more readily obtained on this basis. They are subject to more error, however, as a basis for calculating nutrient intakes. Anyone who has worked with data on food intakes recorded by the subject knows that large errors must have been made by some individuals in keeping the records, although the mean values may be representative of the group as a whole. The low individual records are of most concern, but one does not know to what extent they represent merely omissions from the record rather than dietary inadequacy. Diet histories are very useful as a supplementary measure and as a means of obtaining a general picture of dietary habits, but they hardly suffice in themselves to provide a quantitative picture of food and nutrient intake. Much depends on the skill and experience of the interviewer and the time with each individual. Diet histories can be taken so rapidly and poorly as to result in generally erroneous rather than useful information. Either in taking diet histories or in tabulating food intake records, an increasingly used method is to score the diet for adequacy in terms of specific food groups, viz. two servings daily of fruits and vegetables high in C, one or more servings of fruits and vegetables high in carotene, etc. This system may involve a minimum of error for a diet following the conventional pattern on which the scoring is based. Large errors are possible under other conditions, for there are many different food combinations which can provide an adequate diet.

Much has been written about the relative usefulness of one-, 3- and 7-day records. No generalizations seem worthwhile except to say that a one-day record unaccompanied by any other measure is likely to be meaningless for the individual. Even a week's record has limitations. Seasonal differences have a large influence on the current picture obtained, particularly in rural areas in certain parts of the country. Nutritional status is also a reflection of the previous as well as the current diet. As pointed out by Smith (6) in discussing his survey of maternal nutrition in Holland, "... nutrition, good or bad, is the sum of highly variable quantitative and chronological factors.... The results of any form of dietary inadequacy must vary in accordance with its extent in time...."

The previous discussion is not intended to imply that the shorter methods of collecting and evaluating food data are not useful for various purposes. There are several published reports which show their value (7–9). The studies by Burke and associates showing the influence of nutrition during pregnancy on the condition of the infant at birth are an excellent example. The reports of the survey made under the direction of Harvard workers in New York State following the war, to determine general nutritional status and dietary trends, illustrate how various methods may be used to this end and critically discuss the limitations of each (10, 11). Irrespective of time and cost considerations, the use of more detailed methods has disadvantages in that it may limit the cooperating subjects to a non-representative sample and also result in poor cooperation in the actual recording of data. While contacts by the nutritionists with the subjects both before and during the study, in which its importance is explained, helps greatly in obtaining the desired cooperation, they also may stimulate the subjects to consume a better than usual diet.

Whatever method of arriving at food intakes is

employed, the use of appropriate sampling methods to obtain a representative picture of the population under study is basic. So also is the use of appropriate statistical methods in assessing the significance of the results. These requirements have frequently not been met.

In this talk the limitations of various dietary survey methods have been stressed because it seems important that they be borne in mind in interpreting the conclusions from published results. The discussion should indicate that there is a large background of knowledge and experience of which advantage can and should be taken in making future surveys more reliable and useful. Otherwise, a waste of effort and funds will result and erroneous conclusions will be added to the literature. The effective investigator will be the one who fully understands the advantages and limitations of the various procedures, chooses the one best suited to his specific objective and interprets his results accordingly.

There should be periodic group conferences of workers in the field, such as the U.S.D.A. "Conference on Study of Food Intake," held in October 1948, and the one held at the Children's Fund of Michigan last November dealing with the evaluation of methods of studying the relation of nutrition to maternal and infant health. Such conferences provide the opportunity for the critical comparison of procedures and data. They can also serve to promote joint activities to test the correspondence of the data obtained with a given procedure in the hands of different workers. The results of their deliberations and joint activities should be published to enable others to take advantage of them. By making use of past experience and by critical group examination of current activities, dietary surveys can continue to contribute largely to the advancement of nutrition. Certainly, the methods have limitations but what other method concerned with nutritional status does not?

With respect to the general topic of this symposium, it is clear that dietary records should accompany the appraisal of nutritional status by more direct methods, where possible. Dietary data should help interpret the nutritional significance of the physical signs of deficiency, which are mostly non-specific. They should also aid in clarifying the meaning of various nutrient blood levels since some of them are directly related to current dietary intakes.

In the present situation there is a need for further coordinated studies in which the 3 measures are used with the same subjects, in the interest of evaluating the significance and usefulness of the data of each. Such studies should serve to establish more reliable criteria for evaluating nutritional status by each method. Such continuing research would promote what Dr. King, in a symposium on nutrition surveys in the Federation program 5 years ago, referred to as the most seriously needed study, namely, "to find, through long periods of time, the degree of correlation that exists between the health records of individuals and their dietary habits."

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STANDARDIZATION OF DEFINITIONS AND SYMBOLS IN RESPIRATORY PHYSIOLOGY

On April 19, 1950, a group of physiologists met in Atlantic City to discuss the possibility of establishing a systematic set of symbols for use in teaching and research publications relating to respiratory physiology. The need for such a set of symbols is very great. During the last few years, a number of mathematical generalizations have been developed to describe respiratory processes and these generalizations now form the framework for research and instruction in respiratory physiology in medical schools, clinical laboratories and military establishments throughout the country. While there is general agreement among physiologists concerning the important variables and equations, there has been no effort to reach agreement on definitions and symbols for textbooks or for research publications. Students trained with one set of symbols are bewildered by the multitude of different terminologies in the current literature. Even experts in the field find difficulty in deciphering the equations published by their colleagues from other laboratories.

The discussions at Atlantic City were concerned with two main problems: *a*) definition of lung volumes and *b*) recommendation of a set of symbols which would provide uniformity for the literature without sacrificing flexibility desired for development of new ideas and new equations. The following definitions and symbols represent the fruit of these discussions and they are presented here with the hope that authors and editors will use them as a reference source to achieve uniformity and clarity in terminology in the field of respiratory physiology.

SUBDIVISIONS OF THE LUNG VOLUME

The proposed definitions of lung volume are shown diagrammatically in figure 1. The primary subdivisions of the lung volume shown at right apply to all levels of respiratory effort and contain no overlapping volumes. The 'capacity' terms at left each include two or more of the primary subdivisions and are specially convenient for clinical applications where gasometric measures are most simply made from the expiratory position of the resting patient.

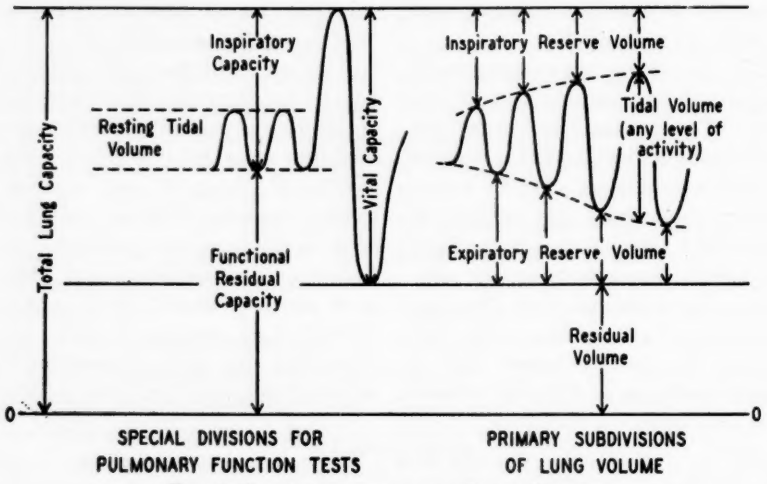

Fig. 1. Subdivisions of the Lung Volume

All gas volumes corrected to body temperature, pressure and saturated with water vapor BTPS. Dimensions to be specified in each publication (e.g., liters, ml., cu. in., etc.). See table 1 for comparison with the various terminologies now in the literature.

Table 1 contains a comparison of the proposed definitions with various current terminologies. The references have been taken from eight widely used textbooks or handbooks and provide a sample of the diverse definitions now in the literature. It will be noted that use of the term 'air' to denote volume is avoided in the new terminology. The terms 'complemental air' and 'supplemental air'

are replaced by 'inspiratory reserve volume' and 'expiratory reserve volume' which have functional meanings and are therefore more easily

TABLE 1. SUBDIVISIONS OF THE LUNG VOLUME: COMPARISON OF PROPOSED TERMINOLOGY WITH PREVIOUS TERMINOLOGIES

PROPOSED TERM	PREVIOUS TERMS WITH REFERENCES
I. Inspiratory reserve volume	Complemental air (3, 4, 7) Complementary air (5) Complemental air minus tidal air (1, 2) Inspiratory capacity minus tidal volume (6)
II. Expiratory reserve volume	Supplemental air (4, 7) Reserve air (1, 2, 3, 5, 7, 8)
III. Tidal volume	Tidal air (All references except 6)
IV. Residual volume	Residual air (1, 2, 4, 5, 7, 8) Residual capacity (6)
V. Vital capacity	Vital capacity (All references)
VI. Inspiratory capacity	Complemental air (1, 2) Complementary air (8)
VII. Functional residual capacity	Functional residual air (1, 2, 7) Midcapacity (1, 4) Normal capacity (3)

remembered. The old controversy as to whether 'complemental air' should include tidal 'air' has been avoided by introducing the term 'inspiratory capacity' to denote the sum of a resting tidal volume and the inspiratory reserve volume.

A SET OF SYMBOLS FOR USE IN RESPIRATORY PHYSIOLOGY

In designing a set of symbols for general use in respiratory physiology it was our aim to provide a systematic method requiring as few special definitions as possible. The method of choice was to adopt single large capital letters to denote general respiratory variables such as pressure, volume, concentration etc., and to modify each variable with one or more small type symbols denoting location, molecular species or special

TABLE 2. SYMBOLS FOR RESPIRATORY PHYSIOLOGY

	SYMBOL	DEFINITION. DIMENSIONS AND CONDITIONS MUST BE SPECIFIED IN EACH PUBLICATION
I. General variables	V	Gas volume in general. Pressure, temperature and percentage saturation with water vapor must be stated.
	$\dot{V}$	Gas volume per unit time
	P	Gas pressure in general
	F	Fractional concentration in dry gas phase
	$\dot{Q}$	Volume flow of blood
	C	Concentration in blood phase
	f	Respiratory frequency—breaths per unit time
	R	Respiratory exchange ratio in general (volume CO_2/volume O_2)
	D	Diffusing capacity in general (volume per unit time per unit pressure difference)
II. Symbol for the gas phase (SMALL CAPS)	I	Inspired gas
	E	Expired gas
	A	Alveolar gas
	T	Tidal gas
	D	Dead space gas
	B	Barometric
III. Symbol for the blood phase	b	Blood in general
	a	Arterial (exact location to be specified in each publication)
	v	Venous (exact location to be specified in each publication)
	c	Capillary (exact location to be specified in each publication)
IV. Special symbols and abbreviations	$\bar{X}$	Dash above any symbol indicates a mean value
	$\dot{X}$	Dot above any symbol indicates a time derivative
	s	Subscript to denote the steady state
	STPD	Standard temperature, pressure, dry (0°C., 760 mm. Hg)
SMALL CAPS	BTPS	Body temperature, pressure, saturated with water
	ATPD	Ambient temperature, pressure, dry
	ATPS	Ambient temperature, pressure, saturated with water

TABLE 3. RESPIRATORY EQUATIONS: EXAMPLES OF USE OF NEW TERMINOLOGY

I. Calculation of oxygen consumption or carbon dioxide production from analysis of volume and composition of expired gas while breathing any mixture of oxygen and carbon dioxide in nitrogen or other neutral gas.

$$\dot{V}_{O_2} = \dot{V}_E \frac{[F_{I_{O_2}}(1 - F_{E_{CO_2}}) - F_{E_{O_2}}(1 - F_{I_{CO_2}})]}{(1 - F_{I_{O_2}} - F_{I_{CO_2}})} \quad (1)$$

$$\dot{V}_{CO_2} = \dot{V}_E \frac{[F_{E_{CO_2}}(1 - F_{I_{O_2}}) - F_{I_{CO_2}}(1 - F_{E_{O_2}})]}{(1 - F_{I_{O_2}} - F_{I_{CO_2}})} \quad (2)$$

where all volumes are corrected to STPD. The quotient of *equations 2* and *1* yields the respiratory exchange ratio for the expired gas.

$$R_E = \frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}} = \frac{[F_{E_{CO_2}}(1 - F_{I_{O_2}}) - F_{I_{CO_2}}(1 - F_{E_{O_2}})]}{[F_{I_{O_2}}(1 - F_{E_{CO_2}}) - F_{E_{O_2}}(1 - F_{I_{CO_2}})]} \quad (3)$$

For the special case where carbon dioxide may be considered negligible in the inspired gas, these equations reduce to simpler forms. If measurements are made in the steady state then $R_E = R_S$ = metabolic respiratory quotient.

II. Calculation of the pressure of oxygen in alveolar gas while breathing any mixture of oxygen with neutral gas at any barometric pressure.

$$P_{A_{O_2}} = F_{I_{O_2}}(P_B - P_{A_{H_2O}}) - P_{A_{CO_2}}\left[F_{I_{O_2}} + \frac{(1 - F_{I_{O_2}})}{R_A}\right]$$

This equation is of special importance in determining equivalent altitudes and oxygen specifications for aircraft. It is frequently called the 'alveolar' equation although it applies equally to any part of expired gas which has undergone respiratory exchange.

TABLE 3—*Continued*

III. The relations between alveolar ventilation and metabolism

$$\dot{V}_A = \frac{\dot{V}_{CO_2}}{F_{A_{CO_2}}} = \frac{R_A}{F_{A_{CO_2}}} \cdot \dot{V}_{O_2} = (P_B - P_{A_{H_2O}}) \cdot \frac{R_A}{P_{A_{CO_2}}} \cdot \dot{V}_{O_2}$$

where

$$\dot{V}_A = (V_T - V_D)f$$

IV. The Bohr Equation for the respiratory dead space to any gas x

$$V_{D_x} = \frac{(F_{E_x} - F_{A_x})}{(F_{I_x} - F_{A_x})} \cdot V_T$$

where all volumes are corrected to BTPS.

V. Fick's Principle becomes

$$\dot{V}_{O_2} = \dot{Q}b(Ca_{O_2} - C\bar{v}_{O_2})$$

VI. The ratio between alveolar ventilation and blood flow through the lungs (cardiac output) in the steady state

$$\frac{\dot{V}_A}{\dot{Q}b} = Rs(P_B - P_{A_{H_2O}}) \frac{(Ca_{O_2} - C\bar{v}_{O_2})}{P_{A_{CO_2}}}$$

VII. The diffusing capacity of the lungs to oxygen as calculated by the Bohr method

$$D_{L_{O_2}} = \frac{\dot{V}_{O_2}}{P_{A_{O_2}} - \bar{P}c_{O_2}}$$

where the mean oxygen pressure in capillary blood ($\bar{P}c_{O_2}$) is calculated from graphical integration of the oxygen dissociation curve.

conditions. The following conventions were adopted for symbols denoting location and molecular species:

1. Localization in the gas phase is represented by a small capital letter immediately following the principal variable. Thus pressure in *alveolar* gas is represented by P_A.
2. Localization in the blood phase is represented by a lower case letter immediately following the principal variable. Thus pressure in the *arterial* blood is represented by Pa.
3. Molecular species is denoted by the full chemical symbol to be printed in small capital letters immediately following the principal variable.

Thus pressure of carbon dioxide is represented by P_{CO_2}.

4. When specification of *both* location and molecular species is required then the first modifying letter will be used for localization and the second for species. Thus the pressure of carbon dioxide in alveolar gas is represented by $P_{A_{CO_2}}$. In this case, the chemical symbol appears as a subscript.

The full list of symbols which were discussed and approved by the group at Atlantic City is shown in table 2. Some of the commonly used respiratory equations are represented in terms of the new symbols in table 3.

NOTES ON THE USE OF THE PROPOSED SYMBOLS

It is hoped that authors employing the proposed system of symbols will provide a list of definitions similar to those of table 2 as an integral part of each manuscript. We wish to emphasize that the symbols listed in table 2 are sufficient only to describe the principal *existing* respiratory equations, samples of which are illustrated in table 3. New symbols are easily added to the list without altering the method of symbolization. For example, it may be useful to consider separately that part of the total pulmonary blood flow which is effective in the exchange of gases. Suitable subscripts for this purpose (e.g. $\dot{Q}_{L_e}$, to denote effective flow through lungs) should then be included in the list.

No special difficulties are anticipated in connection with printing of the proposed symbols. However, the proposed notation is not adapted to the typewriter. This is a disadvantage but, in our opinion, should not be a deciding factor in the choice of symbols. Several sets of symbols have been devised which were 'tailor-made' for the typewriter but none of them provide a logical system which is easy to learn and adaptable to future developments in the field.

The following have agreed to follow the above terminology and system of notation wherever possible in their future teaching and research publications.

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